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Director: Prof. Dr. Saleh M. Ibrahim

Population Differences In Genetic Susceptibility To Psoriasis

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Yosra Bejaoui

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First referee: Prof. Dr. Saleh M. Ibrahim
Second referee: Prof. Jens Mittag
Chairman: Prof. Nobert Tautz
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I hereby declare that the thesis entitled “Population differences in genetic susceptibility to psoriasis“ has been carried out in the Institute for Experimental Dermatology, University of Lübeck, Germany under the supervision of Prof. Dr. Saleh Ibrahim.

I declare that I composed this thesis and that the work contained herein is my own except where clearly stated otherwise, and that this work has not been submitted for any other degree elsewhere.

// Abstract

Psoriasis is a chronic inflammatory disorder of the skin, with genetic factors reportedly involved in the disease pathogenesis. Numerous studies have been conducted to investigate the genetic associations in psoriasis however reported candidate genes and associated loci were mainly in the European and Asian populations.

Here, I report for the first time a study of psoriasis in an Egyptian population. Three main aspects were investigated: the identification of susceptibility variants through a genome-wide association study, the identification of a specific gene involved in the pathogeneses of psoriasis vulgaris in affected cases of two independent Egyptian families and finally we studied the involvement of mitochondria in the pathogenesis of psoriasis through the whole mitochondria sequencing analysis.

The genome-wide association study (GWAS) in an Egyptian population was carried through a two-stage case-control design. In the first stage, we carried out a genome-wide association analysis using the Infinium® GlobalScreeningArray-24v1.0, on 253 cases and 449 control samples of Egyptian descent. In the second stage, we selected 26 single-nucleotide polymorphisms (SNPs) for replication in an additional 321 cases and 253 controls. In concordance with the findings from previous studies on other populations, we found a genome-wide significant association between the major histocompatibility complex (MHC) and the disease at rs12199223 (P_{comb}= 6.57×10^{-18}) and rs1265181 ($P_{comb=}$1.03× 10^{-10}). Additionally, we identified a novel significant association with the disease at a locus, 4q32.1 (rs12650590, P_{comb}=4.49× 10^{-08}) in the vicinity of gene GUCY1A3, and multiple suggestive associations, e.g. rs10832027 ($P_{comb=}$7.28× 10^{-06}) and rs3770019 ($P_{comb=}$1.02× 10^{-05}). This suggests the existence of important inter-ethnic genetic differences in psoriasis susceptibility without disregarding the difference in the environment between the populations. Further studies are necessary to elucidate the downstream pathways of the new candidate loci.

The whole-exome sequencing of the two independent Egyptian families resulted in the discovery of two novel nonsense mutations p.G1054D, p.R1257L in the laminin alpha, 4 gene *LAMA4* in psoriasis vulgaris affected members of family I and II respectively. Followed by a targeted resequencing for a region of 784,424 bp covering the *LAMA4* and *TRAF3IP2* region at chromosome 6q21 by screening 100 psoriatic and 92 control Egyptian samples. This showed the absence of the identified mutations in the screened samples suggesting that the

identified mutations are family specific. Further confirmation was carried by the gene-wise analysis using SKAT showing no significance in the *LAMA4*. In concordance with previous data rs13190932, a SNP in *TRAF3IP2* 662 Kb distal from LAMA4, was associated with psoriasis in the German population, resulted in a suggestive significance with a *P* value of 0.023 in 524 cases and 808 control genotyped samples.

In parallel, we investigated the effect of mitochondria variants on psoriasis sporadic cases. We sequenced 107 healthy Egyptians and110 psoriatic cases. The distribution of phylogenetically combined haplogroups showed a tendency of increase in the number of haplogroup M-C, U-K in addition to R and J-T in cases comparing to controls. χ^2 tests in each haplogroup between cases and controls showed a significance in haplogroup N with a *P*-value of 0.05, N haplogroup was under-represented in the cases suggesting a protective mechanism. Our results of the SNPwise analysis using Fisher exact test resulted in 6 different variations with nominal significance. Two of these variations are G1719A and A3105C in Mt-RNR2 with *P*value of 0.01 and 0.0006 respectively. Two located in the D loop at position m.16292 and m.16359 with a *P*-value of 0.04 and 0.009 respectively. In addition a non-synonymous variation at m.12346 located in MT-ND5 and another at m.3705 at MT-ND1. Further investigation of the function of these variations needs to be carried out with an increase in the number of samples in the replication phase to confirm the results.

ZUSAMMENFASSUNG

Psoriasis ist eine chronisch entzündliche Erkrankung. Die Genese der Erkrankung ist nicht geklärt, jedoch konnte der Einfluss genetischer Faktoren in vielen Studien gezeigt werden. Diese Studien beziehen sich jedoch vor allem auf die europäische und die asiatische Population.

In dieser Studie erforschten wir die genetischen Grundlagen der Psoriasis vulgaris in der ägyptischen Bevölkerung. Dabei führten wir drei separate Untersuchungen durch: (i) die Identifikation von mit Psoriasis assoziierten Varianten in einer genomweiten Assoziationsstudie (GWAS), (ii) die Identifikation von spezifischen Genen durch Exom-Sequenzierung betroffener Familienmitglieder in zwei unabhängigen ägyptischen Familien und (iii) den Einfluss mitochondrialer Mutationen in der Pathogenese der Psoriasis durch mitochondriale Sequenzierung.

Die GWAS wurde in zwei Schritten durchgeführt. Dabei führten wir zunächst eine GWAS mit 253 Psoriasis- und 449 ägyptischen Kontrollproben auf dem Infinium® GlobalScreeningArray-24v1.0 durch. Im zweiten Schritt wählten wir dann 26 Einzelnukleotid-Polymorphismen (single-nucleotide polymorphisms, SNPs) aus dem ersten Schritt aus, die wir in weiteren 321 Psoriasis- und 253 Kontrollproben replizierten. In Einklang mit Ergebnissen aus vorherigen Studien konnten wir eine genomweit-signifikante Assoziation mit dem „major histocompatibility complex“ (MHC)-Lokus an den Stellen rs12199223 (P_{comb}= 6.57×10^{-18}) und rs1265181 ($P_{comb=}1.03\times 10^{-10}$) finden. Zusätzlich identifizierten wir eine neue signifikante Assoziation mit der Erkrankung des Locus 4q32.1 (rs12650590, $P_{comb}=4.49\times 10^{-08}$) in der Nähe des Gens GUCY1A3 sowie multiple weitere Assoziationen wie beispielsweise rs10832027 ($P_{comb=}7.28\times 10^{-06}$) und rs3770019 ($P_{comb=}1.02\times 10^{-05}$). Diese Mutationen implizieren, dass es interethnische Unterschiede in der Suszeptibilität für Psoriasis gibt. Zukünftige Studien werden die Signalwege, in denen diese neu identifizierten Gene eine Rolle spielen, weiter beleuchten.

Die Sequenzierung des Exoms von zwei unabhängigen ägyptischen Familien, in denen jeweils mehrere Familienmitglieder unter einer Psoriasis litten, ergab die Identifizierung der beiden Nonsense-Mutationen p.G1054D, p.R1257L im Laminin alpha 4-Gen (*LAMA4)*, welche in allen erkrankten Familienmitgliedern, jedoch in keinem der gesunden Familienmitgliedern auftraten. Im nächsten Schritt sequenzierten wir eine 784,424 Basenpaare umfassenden Abschnitt um die *LAMA4* und *TRAF3IP2*-Region auf dem Chromosom 6q21 in 100 Psoriasis und 92 Kontrollproben. Hierbei ließen sich die in den Familien gefundenen Mutationen weder in den Psoriasis- noch in den Kontrollproben nachweisen. Dies zeigt, dass es sich um familienspezifische Mutationen handelt. Weitere Bestätigung ergab sich durch eine Gen-bezogene Analyse mithilfe einer „SNP-set (Sequence) Kernel Association Test“ (SKAT)-Analyse, wobei sich keine Signifikanz im LAMA4-Gen ergab. In Einklang mit früheren Studien konnte der SNP rs13190932 im *TRAF3IP2*-Gen, welches 662 Kilobasen vom LAMA4-Gen entfernt liegt, gefunden werden (p=0,023).

Als letztes untersuchten wir das Auftreten von Mutationen im mitochondrialen Genom bei Psoriasispatienten. Dazu sequenzierten wir das mitochondriale Genom von 110 ägyptischen Psoriasispatienten und 107 gesunden ägyptischen Kontrollen. Die Untersuchung der Haplogruppen zeigte ein höheres Vorkommen der kombinierten Haplogruppen M-C, U-K und J-T in den Psoriasispatienten im Vergleich zu den Kontrollen. Mithilfe des χ^2-Tests konnte zudem eine signifikante Assoziation mit Haplogruppe N zeigen (p=0,05). Haplogruppe N war in den Psoriasispatienten jedoch unterrepräsentiert, was auf eine protektive Eigenschaft dieser Haplogruppe hindeutet. Die Analyse einzelner SNPs zeigte sechs verschiedene Mutationen auf. Dazu gehören G1719A (p=0,01) und A3105C (p=0,0006) im Mt-RNR2-Gen. Zwei weitere SNPs lagen im D-loop an Position m.16292 (p=0,04) und m.16359 (p=0,009). Zudem fanden sich die nichtsynonymen Mutationen m.12346 im MT-ND5-Gen und m.3705 im MT-ND1-Gen. Zukünftige Studien werden diese Mutationen in einer größeren Kohorte validieren und die funktionellen Konsequenzen dieser Mutationen untersuchen.

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Abbreviation

ATP	Adenosine triphosphate
CCL20	CC-chemokine ligand 20
CI	Confidence intervals
CD	Crohn's disease
CsA	Cyclosporin A
CVD	Cardiovascular Disease
DCs	Dendritic cells
DM	Diabetes mellitus
Emmax	Efficient Mixed-Model Association eXpedited
GPP	Generalized pustular psoriasis
GWAS	Genome-wide association study
HaCaT	Immortalized human keratinocyte cell line
HLC	Human leucocyte complex
HWE	Hardy-Weinberg equilibrium
IBS	Identity by state
IFN	Interferon
IL	Interleukin
MHC	Major histocompatibility complex
mtABs	Antimitochondrial antibodies
mtDNA	Mitochondrial DNA
mtSNP	Mitochondrial single nucleotide polymorphism
NF-κB	Nuclear factor kappa B
NK	Natural killer
OXPHOS	Oxidative phosphorylation
PASI	Psoriasis area and severity index
PCA	Principal component analysis
P_{comb}	P value combined
PCR	Polymerase chain reaction
pDCs	plasmacytoid DCs
Pi	Priority index
PsA	Psoriatic Arthritis
PsV	Psoriasis Vulgaris
ROS	Reactive oxygen species
SCID	Severe combined immunodeficiency
SNPs	Single-nucleotide polymorphisms
TH1	T helper cell type 1
TNF	Tumor necrosis factor
WES	Whole exome sequencing
LD	Linkage disequilibrium
χ^2	Chi-square test

Introduction

1. History of Psoriasis

Psoriasis (OMIM 177900) was first described by Hippocrates (460-377BC), when all that was known about the disease that it caused a terrible itch to the skin, referred to by the Ancient Greeks as psora, “to itch” (Fox, 1915). At the time, psoriasis was thought to be the same condition as leprosy and people with the condition were considered outcasts (Cowden and Van Voorhees, 2008). Robert Willan (1757-1812) made the first accurate clinical description of psoriasis in 1809; he called the disease lepra vulgaris, consequently causing confusion with leprosy. The disease was not fully acknowledged as a distinct clinical entity until 1841 when Ferdinand von Hebra (1816-1880) named the disease after the original Greek word used to describe it “Psora”.

2. Epidemiology of Psoriasis

Psoriasis has a prevalence of 2-3% in the general population (Parisi et al., 2013). However, this figure varies according to population ethnicity, gender, and age. The prevalence of psoriasis is studied in a number of populations, including countries from Europe and Asia, as well as the USA, Egypt, and Tanzania. It has been observed that there is a positive correlation between distance from the equator and prevalence of psoriasis (Parisi et al., 2013). Prevalence of psoriasis in these studies ranged from 0-3%. The lowest estimates generally come from the Asian and African countries where no more than 0.5% of people have the disease. Higher prevalence is found in Europe and North America, where most of the studies indicate 1.5-2.5% of people are affected. Data on epidemiology in Egypt is scarce, Abdel-Hafez and his colleagues reported a prevalence of 0.19 % in their survey on 8008 rural inhabitants of all ages and both genders from a representative of three villages in Assiut Governorate, Upper Egypt (Abdel-Hafez et al., 2003).

Most studies report that there is no difference in the gender distribution where men and women are equally affected (Braathen et al., 1989). However, some studies report discrepancies; most notably, a study in the Australian population reports that 8.9% of men compared to 4.5% of women have psoriasis (Plunkett et al., 1999). A higher prevalence in men has also been reported in China where 0.17% of men compared to 0.12% of women are affected (Shao et al., 1987). In contrast, studies in USA and Norway found higher psoriasis prevalence in women (Falk and Vandbakk, 1993; Stern et al., 2004). However, these studies tend to draw their findings from limited samples size and more recent large-scale studies,

particularly those in the Taiwan, USA and UK populations, have found no difference in prevalence between the sexes (Gelfand et al., 2005; Tsai et al., 2011).

Discrepancies were found also in reporting the correlation of psoriasis with age. Some studies have shown that psoriasis prevalence is positively correlated with age, with a peak around 50-60 years, after which the rate steadily decreases (Gelfand et al., 2005; Tsai et al., 2011). In contrast, studies from Norway and Spain amongst others report a peak around 20-30 years of age (Falk and Vandbakk, 1993) (Ferrándiz et al., 2002).

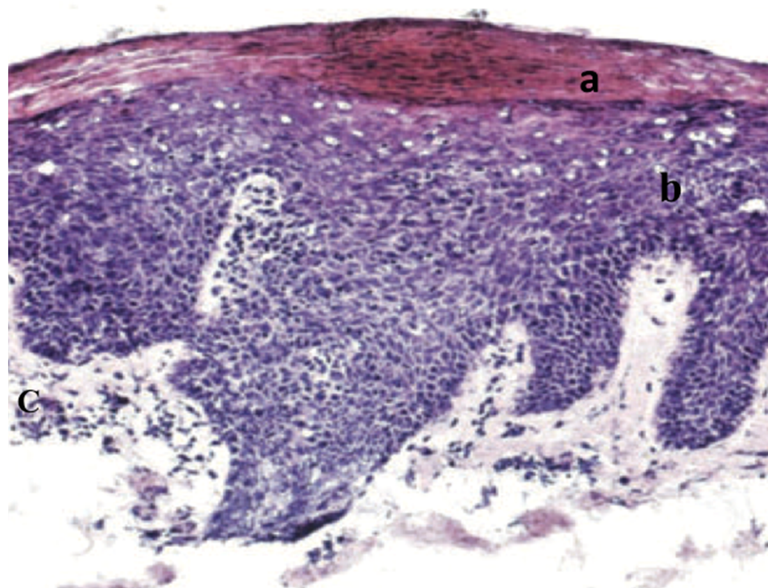
3. Clinical and Histology of Psoriasis

The disease is manifested as raised, well-demarcated, erythematous oval plaques with adherent silvery scales. Clinical features are explained by the growth and dilation of superficial blood vessels causing the redness and equally hyperplasia of the epidermis. The cell cycle time of hyperproliferating psoriatic keratinocytes is short. While maturation and shedding of keratinocytes take 26 days in normal epidermis, it occurs in 4 days in psoriatic epidermis. In the normal epidermis, basal keratinocytes divide approximately every 13 days, with the majority of this time spent in the G1 phase of the cell cycle. The maturation and subsequent shedding of these cells take approximately 26 days. About 10% of basal keratinocytes are cycling in normal skin, whereas this value rises to 100% in the lesional psoriatic skin. Epidermal growth occurs in a pattern termed "psoriasiform'' hyperplasia, which describes by elongated rete pegs, thickening (acanthosis), and differentiation changes. In the psoriatic epidermis, keratinocytes proliferate and mature rapidly so that terminal differentiation, normally occurring in granular keratinocytes and then squamous corneocytes, is incomplete. Hence, squamous keratinocytes aberrantly retain intact nuclei (parakeratosis) and release a few extracellular lipids that normally cement adhesions of corneocytes. Accordingly, poorly adherent stratum corneum is formed and this results in the characteristic scale or flakes of psoriasis lesions (Nestle et al., 2009a).

The inflammatory infiltrates consists mainly of dendritic cells, macrophages, T cells and neutrophils in the dermis, with some T cells in the epidermis. The redness of the lesions is due to increased numbers of tortuous capillaries that reach the skin surface through a marked thinned epithelium. However, even with this characteristic array of cellular changes, it is necessary to exclude skin infection by yeast or fungi, since the immune reaction to these microbes can produce a virtually identical histological picture.

Histological examination with hematoxylin and eosin staining demonstrates acanthosis, Munro microabscesses known as an accumulation of neutrophils in the epidermis, alternating neutrophils (isolated neutrophils within the stratum corneum) and parakeratosis in the stratum corneum and infiltration of mononuclear cells in the dermis and epidermis (Greb et al., 2016a).

Figure 1: Haematoxylin and eosin–stained section from a chronic psoriatic plaque The lesion displays (a) retention of nuclei in the stratum corneum (b) marked thickening of the epidermis (c) with a brisk cellular infiltrate in the upper papillary dermis. Modified from : (Greb et al., 2016b)



4. Classifying Psoriasis

Psoriasis is rarely life-threatening in itself with only severe cases conferring a 3-4 year reduction in the life expectancy of patients (Gelfand et al., 2007). The majorities of psoriatic patients have mild or moderate symptoms, limited body coverage and most live to full life expectancy (Gelfand et al., 2004). However, psoriasis has a significant negative impact on quality of life with patients experiencing varying levels of discomfort, which can interfere with daily routines(Basavaraj et al., 2011). This can have a knock-on effect on income with the high cost of therapy adding to the economic cost (Horn et al., 2007). The disease is usually life-long from the onset, with a small percentage of cases spontaneously resolving.

The most commonly used method for measuring psoriasis disease activity is the psoriasis area and severity index (PASI). PASI is considered the gold standard and was introduced in 1978

(Fredriksson and Pettersson, 1978). It measures psoriasis in three different aspects; erythema (E; redness), induration (I; thickness) and desquamation (D; scaliness). Each aspect is graded on a scale of 0-4, 0: “no involvement”, 1: “slight”, 2: “moderate”, 3: “marked”, 4: “very marked”. These aspects are assessed in four different regions of the body, together with the area of psoriasis coverage (A) as used in the body surface area index.

5. Types of Psoriasis

Psoriasis Vulgaris (PsV)

The most common form of psoriasis is PsV also referred to as chronic plaque psoriasis (CPP). PsV is present in about 90% of psoriatic patients, with typical features including erythema (redness), induration (thickening and rising) and desquamation (peeling) of the skin to form characteristic plaques that are well defined from unaffected regions of the skin. Psoriatic plaques are often covered in silvery-white scales and affected regions of the skin include the scalp, extensor surfaces (elbows and knees), the spinal area of the back and the buttocks (Griffiths and Barker, 2007). Psoriatic plaques can vary in size either covering large areas of skin or smaller localized areas, which are often circular in shape.

Flexural Psoriasis

Intertriginous psoriasis exclusively affects skin folds including behind the ears, under the breast and the intergluteal cleft. Flexural psoriasis usually lacks scaling and appears as smooth areas of very red, shiny lesions. It is estimated to affect about 3-7% of psoriasis patients (Wang et al., 2005).

Psoriatic Erythroderma

Psoriatic erythroderma is an extreme and generalized form of PsV that is more widespread across the body, with coverage of more than 90% (Rosenbach et al., 2010). Symptoms tend to be more aggressive with severe discomfort and patients often reporting being constantly unwell.

Pustular Psoriasis

Pustular psoriasis presents as raised red lesions containing pus, often localized to small regions but also occurring in wider areas over the body (Khan, 1972). The pustules are sterile and are classified as acute or chronic according to the length of time psoriasis appears on the skin. The acute form of pustular psoriasis is also known as generalized pustular psoriasis (GPP) or Von Zumbusch psoriasis after a German dermatologist who first described it. GPP is often associated with fever and is considered to require immediate medical attention. A more chronic annular, ring-shaped form of pustular psoriasis (APP) has also been described. It is

one of the rare forms of the disease but has been reported to be the most prevalent form of psoriasis in children.

Guttate Psoriasis

Guttate psoriasis is notable for the occurrence of numerous small pink/red scaly lesions over wide areas of the body and its limited duration (Griffiths and Barker, 2007). Guttate psoriasis often appears on the trunk and usually develops 2-3 weeks after a Streptococcal throat infection. Despite often the following infection, it is not contagious and usually resolves within a month. Around a third of guttate psoriasis patients go on to develop CPP (Martin et al., 1996). Studies have shown that guttate psoriasis is associated with an early age of onset with adolescents being most affected.

Nail Psoriasis

Nail psoriasis is a specific form of the disease. Reports vary with regards to its incidence amongst psoriasis sufferers with a range of about 50-80% (Salomon et al., 2003). However, there is a high level of incidence amongst patients with psoriatic arthritis (PsA), with about 80% of sufferers also having joint inflammation (Lawry, 2007). Clinical presentations include pitting, which are small holes in the nail caused by loss of keratin.

6. Pathogenesis of Psoriasis

Psoriasis is a multifactorial immune-mediated disease, but the pathophysiology of the condition is not fully understood. It was initially considered a disease with a primary defect in keratinocytes, (Krueger et al., 1984). However, several observations indicated the involvement of the immune system particularly T cells. In 1979, it was reported that cyclosporin A (CsA) improved psoriatic skin eruptions and it was shown that immunosuppressive and T cell targeted therapy improve the disease (Ellis and Krueger, 2001; Lebwohl et al., 2003; Roenigk et al., 1972). Psoriasis is regarded as an autoimmune disease; however, the nature of the autoantigens that trigger T-cell activation remains unclear.

Psoriasis can be induced in healthy skin from psoriatic patients transplanted into severe combined immunodeficiency (SCID) mice, by transferring T cell clones from the same patient (Wrone-Smith and Nickoloff, 1996). Keratinocytes are not regarded as bystanders in the psoriasis lesions. Recent investigations have shown that mice with genetically altered epidermal phenotypes may develop psoriasis-like skin lesions, for example as an effect of overexpressing the transcription factor signal transducer and activation of transcription 3 in keratinocytes, or induced deletion of transcription factors JunB and c-Jun in keratinocytes (Sano et al., 2005; Zenz et al., 2005). Keratinocytes are also important in sustaining and amplifying inflammatory responses through production of cytokines and growth factors

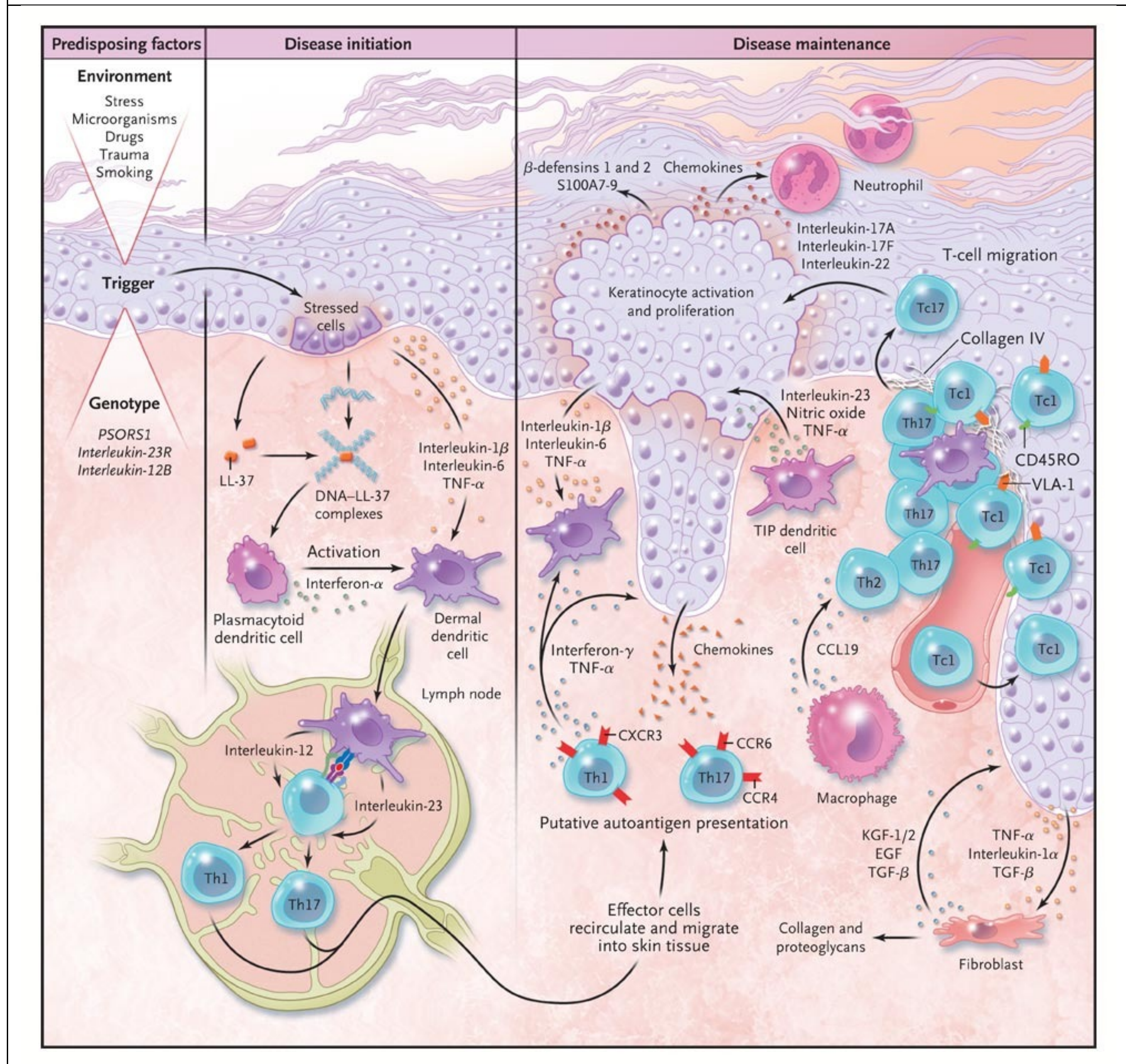
affecting lymphocyte recruitment and activation, for example TNF-α, vascular endothelial growth factor, IL-1, IL-6, IL-15 and IL-18 (Albanesi et al., 2007; Lowes et al., 2014; Nickoloff et al., 2007). The earliest histological event in lesion development is the accumulation of T cells, monocytes and dendritic cells (DCs) around the dermal blood vessels (Ragaz and Ackerman, 1979). Additionally, cytokines and chemokines produced by keratinocytes may control the initial entry of T cells into the skin (Albanesi et al., 2007). The activated T cells show a TH1 type cytokine profile, including TNF-α, IL-12, IL-8, and IFN-γ, which is thought to be a key cytokine in driving the keratinocyte hyperproliferation (Bata-Csorgo et al., 1995; Jackson et al., 1999). Natural killer (NK) T cells and NK cells are also present in psoriasis lesions and may be an important source of IFN-γ as well as other TH1 cytokines in the early initiation phase of lesion formation (Cameron et al., 2002). Recently, an important role for IFN-α produced by plasmacytoid dermal DCs in the initiation of psoriasis lesions has been proposed (Nestle et al., 2005).

One theoretical model for psoriasis pathogenesis is described below (Figure 2), although there are still many unresolved issues in explaining this complex condition (Griffiths and Barker, 2007). Alterations in skin barrier and immune genes enable an abnormal response triggered by yet largely unidentified environmental risk factors or antigen (Nestle et al., 2009a). A complex interplay between the skin epithelium and connective tissue with the innate and adaptive immune system is initiated.

When triggering factors cause skin injury, keratinocytes activate myeloid dendritic cells (DCs) with Toll-like receptors (TLRs), antimicrobial peptide LL37, and plasmacytoid DCs (pDCs) (Nestle et al., 2005). Keratinocytes produce CC-chemokine ligand 20 (CCL20), which attracts myeloid DCs and T17 cells. Activated myeloid DCs stimulate psoriatic T cells producing IL-17 (T17), IFN- γ (Th1, Tc1), and IL-22 (Th22, Tc22). The cytokines released by these cells further stimulate keratinocytes, and the immune circuit is amplified by the feedback cytokines production from keratinocytes increasing the natural turnover of the epidermis and eventually leads to the formation of the characteristic psoriasis skin plaques. IL-23/T17 axis is the dominant pathogenic pathway in psoriasis. It is notable that IL-12/IFN-γ axis potentially suppresses IL-23/T17 axis. IL-19, IL-20, and IL-24 are also upregulated in psoriatic lesions and have biologic effects similar to IL-22 (Zaba et al., 2007; Zheng et al., 2007).

Figure 2: Proposed Schematic diagram of the pathogenesis of psoriasis
Showing the interplay between the genetic and environmental in the initiation of the disease and the interaction of the immune cell with keratinocyte in the disease manifestation

Adapted from:(Nestle et al., 2009b)



7. Comorbidities of Psoriasis

Psoriasis has well-established and emerging associated comorbidities. One of the most conclusive comorbidities is cardiovascular disease. Other associated systemic diseases include Crohn's disease (CD) (Fiorino and Omodei, 2015), the metabolic syndrome (Coto-Segura et al., 2013, p. 2), and lymphoma, although in the last disease it is difficult to determine the effects of psoriasis therapy.

Cardiovascular Disease

Cardiovascular disease (CVD) is another class of disorders that has emerged as a comorbidity of psoriasis. A prospective population-based study of psoriasis patients in the United Kingdom showed that psoriasis confers an independent risk of myocardial infarction and that the relative risk of myocardial infarction is greatest in young patients with severe psoriasis (Gelfand et al., 2006). Subsequently, studies have analyzed the association of psoriasis to a number of CVD disorders including myocardial infarction, occlusive vascular disease, heart failure, ischaemic heart disease, cerebrovascular disease and atherosclerosis (Coumbe et al., 2014). A study in 2004 found an increased risk of ischaemic heart disease and cerebrovascular disease amongst hospital in patients with severe psoriasis (Mallbris et al., 2004).

Crohn's Disease

Studies have suggested an increased prevalence of CD in psoriatic patients (Huang et al., 2012). A later study supported this by reporting that psoriasis was found in 9.6% of CD patients compared to 2.2% in the general population of Blackpool (Lee et al., 1990).

Diabetes Mellitus

There are a number of studies that have established a link between psoriasis and diabetes. An early study in 1986 established a link between psoriasis and diabetes in women (Lindegård, 1986). This was confirmed in a more recent study in the UK which found that diabetes mellitus (DM) had an incidence of 1.92% in patients with psoriasis, compared with 1.32% in those without psoriasis (Brauchli et al., 2008).

Metabolic Syndrome

A comorbidity related to DM is a metabolic syndrome, which is one of a number of disorders that are associated with psoriasis recently. Metabolic syndrome is defined by the World Health Organization's 1999 criteria as the occurrence of one of DM, impaired glucose tolerance, impaired fasting glucose or insulin resistance along with two of the following; high blood pressure, dyslipidemia and high-density lipoprotein cholesterol, obesity, or body mass index or microalbuminuria.

Psoriatic Arthritis

Psoriatic arthritis (PsA) has been established as a disease, independent of both rheumatoid arthritis (RA) and psoriasis since 1948 (Gladman, 2005). It is defined as the occurrence of seronegative spondyloarthropathy with psoriasis. PsA has a prevalence of 0.04-1.2% in the general population, compared with 6-42% in patients with psoriasis (Gottlieb et al., 2008). The majority of PsA cases develop skin symptoms prior to joint involvement, with one study suggesting that this is the case for around 84% of patients.

Cancer

A number of studies have investigated the link between psoriasis and cancer; the data have been inconsistent. Lymphoma had shown the strongest association with psoriasis with one study reporting an approximately 8-fold increase in risk amongst patients being treated with methotrexate or cyclosporine (Margolis et al., 2001).

8. Genetics of Psoriasis

In contrast to Mendelian conditions, where mutations are rare in the population and have a large effect, complex disease such as psoriasis is due to the multiple common alleles that contribute to the susceptibility with modest risk (Lander and Schork, 1994).

Population studies clearly indicate that the incidence of psoriasis is greater among first-degree and second-degree relatives of patients than among the general population (Farber and Nall, 1974). Psoriasis has been extensively studied in multi-generation families supporting the genetic basis for psoriasis. Accordingly, an offspring of two affected parents have a 50% chance of developing psoriasis, however, this decrease to 16% if only one parent is affected. In addition, if psoriasis is present in one child and absent in the parent, there is an 8% chance for that child's sibling to develop psoriasis (Abele et al., 1963). Studies of psoriasis disease concordance among twins showed that the risk of psoriasis is two to three times as high among monozygotic twins as among dizygotic twins (Farber and Nall, 1974).

The heritability, the proportion of variability of a trait attributed to a genetic factor, is believed to range from 60% to 90% in psoriasis (Elder et al., 1994).

Based on this “common disease-common variant” hypothesis, numerous genome-wide linkage scans and association studies have been conducted to investigate the multiples genes that predispose psoriasis (Oka et al., 2012).

Linkage-Based Approaches

The earliest efforts to identify susceptibility loci in psoriasis began by genome-wide scans in families affected by psoriasis using genetic linkage techniques. The linkage is the tendency for two genetic markers to be inherited together within families due to their close proximity on the same chromosome. Markers used are either single nucleotide polymorphisms or microsatellites (Griffiths, 1993). This approach is a family-based approach quite successful in genetic studies of Mendelian diseases. However, in the case of a common complex genetic disorder such as psoriasis, linkage-based approaches are less effective because complex diseases are caused by the accumulation of common alleles in the population with a low penetrance and mild contribution to the disease (Gupta et al., 2014a). All these factors

necessitate that a large number of samples be studied for genetic markers to obtain good statistical power.

Nevertheless this approach had identified at least 10 chromosomal segments (genetic loci) that cosegregate with psoriasis (PSORS1-10); these susceptibility regions, detected by linkage analysis in psoriasis family, include 6p21.3 (PSORS1), 17q25 (PSORS2), 4q (PSORS3), 1q21 (PSORS4), 3q21 (PSORS5), 19p13 (PSORS6), 1p (PSORS7), 16q (PSORS8), 4q31-34 (PSORS9) and 18p11.23. Other than PSORS1, no other major susceptibility locus was consistently replicated by other studies. (Gupta et al., 2014b)

PSORS1

PSORS1 is a 220-kb region in the major histocompatibility complex (MHC) region known as psoriasis susceptibility 1. It is known as the major genetic determinant of psoriasis. It contains 10 known genes (Trembath et al., 1997a). *HLA-Cw0602* allele was first associated with psoriasis by serological typing (Tiilikainen et al., 1980). *HLA-Cw0602* allele confers a 20-fold-increased risk of developing the disease and it is carried in up to 60% of psoriatic patient and 10-15% in the general population (Trembath et al., 1997b). When present in homozygous it has a fivefold-increased risk compared with heterozygous individuals (Gudjonsson et al., 2003). Several studies have been conducted to dissect the mechanism of the *HLA-Cw0602* in the molecular pathogeneses of psoriasis. It has been hypothesized that HLA-Cw6 may have a high binding affinity for one or more psoriasis autoantigens. Arakawa et al found that HLA-Cw6 present a specific melanocyte auto-antigen which is (ADAMTS-like protein 5) to CD8+ T cells (Arakawa et al., 2015). Structural biology studies have shown that the peptide binding groove of HLA-Cw6 has high-affinity for LL-37 (Mabuchi and Hirayama, 2016), a molecule described as a T-cell autoantigen in psoriasis (Lande et al., 2014). The actual causative gene in *PSORS1* locus was extremely challenging to identify due to the extensive linkage disequilibrium within the MHC. The additional suggestive causative genes located in PSORS1 are the corneodesmosin (*CDSN*), which plays a role desquamation of the epidermis (Allen et al., 2005) and coiled-coil α-helical rod protein 1 (*CCHCR1*) is up-regulated in psoriasis affected skin and has a role in keratinocyte proliferation (Oka et al., 1999; Suomela et al., 2003; Tiala et al., 2008).

Although the *PSORS1* locus confers the most risk for psoriasis, it accounts for only 50% of the familial clustering observed in psoriasis (“The International Psoriasis Genetics Study,” 2003).

PSORS2

PSORS2 maps to chromosome 17q25.It was first identified in a study of 8 multiple affected psoriasis north American families by polymorphic microsatellite markers (Tomfohrde et al., 1994). The refinement of *PSORS2* led to the identification of suggestive genes that plays a role in psoriasis pathogenesis such as the solute carrier family 9 isoforms 3 regulatory factor 1 (*SLC9A3R1*) and N-acetyltransferase 9 (*NAT9*) (Helms et al., 2003). *SLC9A3R1* is associated with diverse aspects of epithelial membrane biology and immune synapse formation in T cells, It functions as an antigen recognition molecule and binds to runt-related transcription factor 1 (*RUNX1*). A SNP between SLC9A3R1 and NAT9 was found to lead to loss of RUNX1 binding (Helms et al., 2003).

With the advent of next-generation sequencing a gain-of-function mutation that segregated with psoriasis were identified in caspase recruitment domain family, member 14 *CARD14* by using genomic capture and DNA sequencing (Jordan et al., 2012). *CARD14* identified mutation leads to a constitutive NF-κB activation, enhancing the production of pro-inflammatory cytokines (Berki et al., 2015).

PSORS3

PSORS3 was first mapped to chromosomal region 4q34 in 1996 (Matthews et al., 1996). *PSORS3* harbors the following candidate-genes interferon regulatory factor 2 (*IRF2*) (Foerster et al., 2004), interleukin 21 (*IL21*), Interleukin 12 (*IL12*). *IRF2* is a transcription factor that competitively inhibits the *IRF1*-mediated transcriptional activation of *IFN-α* and *IFN-β* (Hida et al., 2000).

PSORS4

The PSORS4 region maps to chromosome 1q2 (Capon et al., 1999). The Epidermal Differentiation Cluster (EDC) spams this region. EDC is an evolutionary conserved genomic segment that consists of a cluster of genes important for the terminal differentiation of the epidermis (Oh and de Guzman Strong, 2017).

PSORS5

PSORS5 is mapped to 3q21. It was found to be linked to psoriasis by a Swedish study conducted in 1999(Samuelsson et al., 1999). Numbers of candidate-genes were suggested in the *PSORS5* region. Carrier Family 12 Member 8 (SLC12A8) gene was shown to be strongly associated with psoriasis (Hewett et al., 2002). Another identified gene in *PSORS5* region is cystatin A (*CSTA*) identified in a family-based association study (Vasilopoulos *et al.* 2008).

PSORS6

PSORS6 is mapped to chromosome 19p13 (Lee et al., 2000). A gene lying near this region called mucin 16 (*MUC16*) was suggested as a candidate gene. MUC16 is expressed in epithelial cells and possibly plays a role in forming a protective mucous barrier (Hüffmeier et al., 2009).

PSORS7

PSORS7 is mapped to 1p. A protein tyrosine phosphatase, non-receptor type 22 (*PTPN22*), was first associated with psoriasis in 2006 in the German population (Hüffmeier et al., 2006). *PTPN22* codes for a protein involved in T and B lymphocyte receptor signaling. The second candidate-gene is IL23-receptor (*IL23R*) associated with psoriasis in 2007 and has since been replicated in multiple studies (Capon et al., 2007; Wolf et al., 2007; Nair et al., 2008; Smith et al., 2008).

PSORS8

PSORS8 is mapped to chromosome 16q, this was confirmed in the International Psoriasis Genetics Consortium (2003) analyzing 942 affected sibling pairs from 710 pedigrees for 53 polymorphic microsatellites spanning 14 psoriasis candidate regions. (Nair et al., 1997; "The International Psoriasis Genetics Study," 2003). This region was found to be adjacent to a Crohn's disease susceptibility gene, caspase recruitment domain family member 15 (*CARD15*), which was considered a candidate gene for the region.

PSORS9

PSORS9 is mapped to chromosome 4q31. It was identified by a genome-wide scan in a Chinese Hans population in 2002 (Zhang et al., 2002). Interleukin 15 (*IL*15) is so far the only candidate gene suggested in PSORS9. *IL*15 is involved in Th1 cell regulation, hyperproliferation of keratinocyte and mediation of inflammatory response (Nestle et al., 2009b)(Zhang et al., 2007).

PSORS10

PSORS10 is mapped in chromosome 18p11.23 by genome-wide scan, followed by fine mapping, in 9 Finnish families with psoriasis (Asumalahti et al., 2003), no candidate gene was proposed for this region and it was not replicated again in any study.

A summary of PSORS loci identified from linkage studies is provided in Table 1.

Table 1: Loci Associated With Psoriasis (PSORS) from linkage analysis studies		
Locus	Region	Genes
PSORS1	6p21.3	*HLA-Cw6*
PSORS2	17q25.5	*CARD14*
PSORS3	4q34	*IRF-2*
PSORS4	1q21	*EDC*
PSORS5	3q21	*SLC12A8, cystatin A*
PSORS6	19p13	*JunB, MUC16*
PSORS7	1p	*PTPN22, IL23R*
PSORS8	16q	*CARD15*
PSORS9	4q31	*IL15*
PSORS10	18p11	

Association Studies

Linkage studies although they added value in understanding the gene associated with psoriasis; this approach had its limits. For a complex disease such as psoriasis association studies, population-based studies that investigate the association between a disease allele and a particular disease trait in both a case and control population are more powerful. Association studies use a large number of markers and with the advent of genome-wide single nucleotide polymorphism (SNP), microarrays technology and the completion of HapMap project, it became feasible to comprehensively scan the genome (International HapMap Consortium, 2003). In the last years, more than 12 GWAS have been performed on psoriasis reporting 41 genetic loci associated with psoriasis. Most of these studies have confirmed the *HLA-C* association with psoriasis at genome-wide significance ($P < 5 \times 10^{-8}$) and had identified novel, non-HLA associations genes involved in specific inflammatory pathways, including NF-κB and IL23-Th17 (Liu et al., 2008; Zhang et al., 2009; Ellinghaus et al., 2010a; Tsoi et al., 2012).

Populations differences in psoriasis

Analysis revealed in 2009 that 96% of participants in GWAS were of European descent (Need and Goldstein, 2009). The finding derived attention that a much diverse range of populations are to be investigated to avoid the fact that genomic medicine being of benefit to a specific population. Later on, findings indicated that the proportion of individuals included in GWAS who are not of European descent has increased to nearly 20% in 2016. This is a result of studies being done on Asia on populations. The degree to which people of other populations such as African, Latin American and other indigenous population are involved has barely shifted. Figure 3 represents the proportion of participants in GWAS studies in the year 2009

and 2016 showing that in seven years there was an increase in the non-European ancestry representation in these studies.

Psoriasis population studies were mainly conducted on European and Chinese populations. Table 2 represents the primary studies conducted on European and Chinese cohort. As mentioned earlier 41 genetic susceptibility loci for psoriasis established at a genome-wide level of significance ($p < 5 \times 10^{-8}$), of which 36 have been identified in European Caucasians and five in the Chinese population. Common association between the Chinese and European population also has been observed.

Figure 3: The proportion of participants in genome-wide association studies (GWAS) in 2009 and 2016.

Adapted from:(Popejoy and Fullerton, 2016)

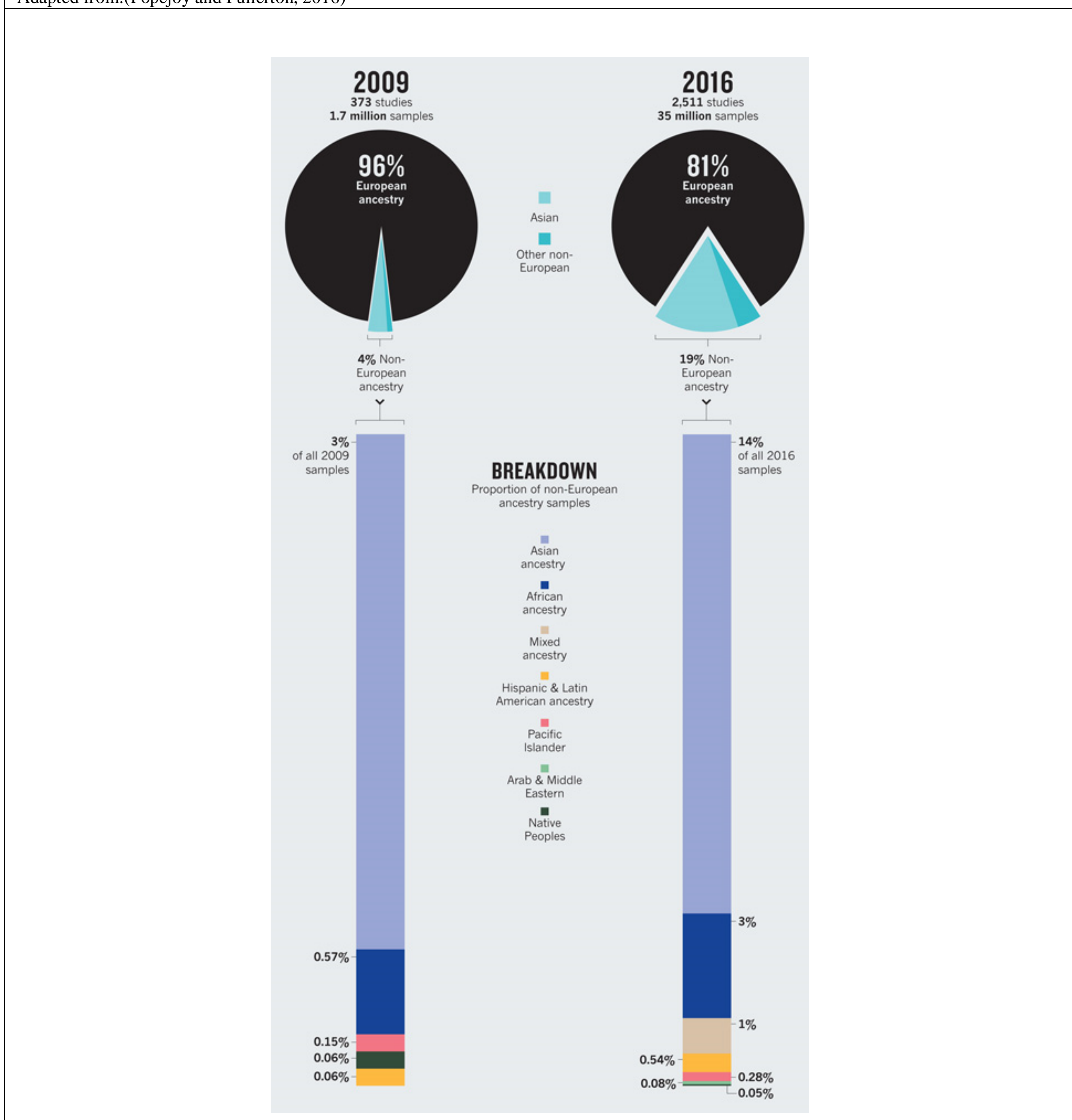


Table 2: GWAS in psoriasis by cohort ethnicity		
	Number of markers genotyped in Discovery phase	Number of Psoriatic samples
European Cohort		
(Liu et al., 2008)	311,398	223
(Nair et al., 2009)	438,670	1,409
(Ellinghaus et al., 2010a)	2,339,118	472
(Hüffmeier et al., 2010a)	1,585,307	609
(Strange et al., 2010)	594,224	2,622
Asian Cohort		
(Zhang et al., 2009)	494,902	1,139

The first GWAS study was carried out an initial discovery cohort from North America. This led to the discovery of *IL12B* association on chromosome 5 and was then replicated in two independent datasets (Cargill et al., 2007). Following that a GWAS in a European cohort of led to the identification of Zinc Finger Protein 313 (ZNF313) as a novel psoriasis susceptibility gene (Capon et al., 2008).

The first GWAS conducted in a Chinese population giving hint to the heterogeneity of the disease between populations replicated the *IL12B* association and identified novel association at four SNPs mapping to the late cornified envelope (*LCE*) gene cluster on chromosome 1. However, no association was found at *ZNF313,* which demonstrates the effect of population ethnicity and demographics in genetic studies (Zhang et al., 2009).

Meanwhile, a Collaborative Association Study of Psoriasis (CASP) consisting of three North American research groups conducted a GWAS in collaboration with the Genetic Association Information Network (GAIN) (Nair et al., 2009). Seven genetic loci showed association with psoriasis with $P < 5 \times 10^{-8}$. Different loci were confirmed such as HLA-C gene involved in *IL23* signaling *(IL23A*, *IL23R*, *IL12B*), *TNIP1*and *TNFAIP3* that act downstream of TNF-alpha and regulate NF-κB signaling and *IL4* and *IL13* involved in the modulation of Th2 immune responses (Nair et al., 2009).
In 2010 two more studies were published simultaneously. The first was a joint collaboration between the Genetic Analysis of Psoriasis Consortium (GAPC) and the Wellcome Trust Case Control Consortium 2 (WTCCC2); the initial analysis confirmed the association of all

previously associated loci up to that point and identified further novel associations at three loci including endoplasmic reticulum aminopeptidase 1 (*ERAP1*) on chromosome 5, interferon induced with helicase C domain 1 (*IFIH1*) on chromosome 2 and TRAF3-interacting protein 2 (*TRAF3IP2*) on chromosome 6. Further associations were identified in the replication cohort such as IL28 receptor antagonist (*IL28RA*) on chromosome 1, v-rel avian reticuloendotheliosis viral oncogene homologue (*REL*) on chromosome 2, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-alpha (*NFKBIA*) on chromosome 14 and tyrosine kinase 2 (*TYK2*) on chromosome 19. The second study was conducted in the German population confirmed associations at *HLA-C*, and *IL12B* and identified an association at TRAF3IP2 which is a gene that encodes a protein involved in IL-17 signaling and which interacts with members of the Rel/NF-κB transcription factor family (Ellinghaus et al., 2010a).

Table 3: Known psoriasis associated gene associated with psoriasis of loci reaching genome-wide significance in the Chinese and Caucasian population.

Population	Gene associated with Psoriasis from GWAS studies
Caucasian	*SLC45A1, TNFRSF9, IL28RA,RUNX, IL23R, REL, B3GNT2, IFIH1, IL13/IL4, EXOC2/IRF4, TRAF3IP2,TNFAIP3, TAGAP, ELMO1, DDX58, KLF4, ZMIZ1,PRDX5, ZC3H12C, ETS1, IL23A/STAT2, NFKBIA, SOCS1, FBXL19, NOS2, STAT3, STAT5A/B, CARD14, STARD6, POLI, MBD2, TYK2, ILF3, CARM1, RNF114, UBE2L3*
Chinese	*PTTG1, CSMD1, GJB2, SERPINB8, ZNF816A*
Caucasian, Chinese	*HLA-C, LCE deletion, ERAP1, TNIP1, IL12B*

Besides these populations no GWAS studies were conducted, only replication of known SNPs were carried in different populations such as Indian and Japanese from Asia (Pitchappan et al., 1989; G et al., 2011; Oka et al., 2013). Regarding north African populations such as Tunisian and Egyptian lack these studies and the main conducted genetic studies in where either Family-based or gene analysis respectively (Ammar et al., 2013; Haase et al., 2014).

Studies of genetic association in ethnically diverse populations will primary help us identifying susceptibility loci specific to the population studied, and define narrower bounds for further analysis of associated regions that are common to multiple populations.

Meta-Analyses and Validation Studies

As the number of published GWAS has grown, the opportunity to perform meta-analysis has increased the power to detect associations with modest effect sizes.

The first GWAS meta-analysis was published consisted of a discovery phase involving data from the Collaborative Association Study of Psoriasis (CASP) study and the German Kiel study. SNP imputation was used to combine the two studies, which gave a cohort of 1,831 cases and 2,546 controls. The top 102 SNPs from this initial analysis were then followed up in a replication phase consisting of 4,064 cases and 4,685 controls from Michigan, Toronto, Newfoundland, and Germany. This resulted in the identification of three new susceptibility loci at F-box and leucine-rich repeat protein 19 (*FBXL19*) on chromosome 16, NFKB Inhibitor Alpha NFKBIA on chromosome 14 and nitric oxide synthase 2 (*NOS2*) on chromosome 17 (Stuart et al., 2010).

Simultaneously with the CASP/Kiel meta-analysis, a large-scale validation study was conducted in the Chinese population (Sun *et al.* 2010). This study again highlighted differences in the genetics underpinning the disease in different populations. To begin with, 61 SNPs showing suggestive association ($P < 10^{-5}$) from the original GWAS study were genotyped in an independent cohort of 4,610 Chinese cases and 5,373 controls. This yielded 3 loci with the genome-wide significant association; the previously discovered *TNIP1* and the novel genes gap junction protein beta 2 (*GJB2*) on chromosome 13 and pituitary tumor-transforming 1 (*PTTG1*) on chromosome 5. Twenty more SNPs with $P < 0.05$ from this first replication analysis were taken forward for genotyping in a second replication cohort of 2,024 cases and 5,495 controls. In addition to the loci mentioned earlier, *ERAP1*, a novel locus for the Chinese population, CUB and Sushi multiple domains 1 (*CSMD1*) on chromosome 8, serpin peptidase inhibitor member 8 (SERPINB8) on chromosome 18 and zinc finger protein 816 (*ZNF816A*) on chromosome 19, were identified. To further assess the association these loci on other populations, they were analyzed in four further replication cohorts consisting of Chinese Uygur, German, and US samples. None of the loci reached the genome-wide threshold in these cohorts, demonstrating the specificity of *CSMD1*, *ERAP1*, *SERPINB8* and *ZNF816A* for the Chinese Han population.

The most recent meta-analysis was a collaboration between Kiel, CASP and GAPC/WTCCC2 published GWAS datasets, together with two independent datasets of European samples from

the Psoriasis Association Genetics Extension (PAGE) and GAPC; (Tsoi *et al.* 2012). The independent datasets were genotyped using the Immunochip custom array, with imputation increasing the number of SNPs analyzed to 111,236. Not only did this meta-analysis replicate all but two (*PRDX5* and *ZMIZ1*) of the previously associated loci at genome-wide significance up to that point, but it also added 15 novel loci, the largest number reported for psoriasis to date.

After the Immunochip study, the number of confirmed loci currently stands at 41. Thirty-one of these have been identified in the European population, five are specific to the Chinese population and five overlaps. Collectively, these loci are estimated to account for around 22% of the total heritability of psoriasis, indicating that there are still a lot of susceptibility variants to discover (Tsoi *et al.* 2012).

9. Mitochondria and Psoriasis disease

The mitochondria are double membrane-bound organelles found in most eukaryotic organism. Their numbers in a cell vary depending on the organism, tissue or cell type. For example, red blood cells lack mitochondria where liver cells can contain around 2000 (Henze and Martin, 2003). A mitochondrion contains an outer and inner membrane composed of phospholipid bilayers and proteins (Alberts, 2002). The main function of mitochondria is generating energy as adenosine triphosphate (ATP) through oxidative phosphorylation (OXPHOS) process. In addition, it plays a role in heme synthesis, apoptosis, and calcium homeostasis. In mitochondria, metabolites are generated through the breakdown of carbohydrates, proteins and fatty acids are then used in the citric acid cycle and OXPHOS to create a proton gradient that produces ATP via ATP synthase. As a result defects at different levels can lead to diminished mitochondrial function and consequently to lower energy production (Wallace et al., 1999). Both mitochondrial and nuclear DNA encode the OXPHOS subunits proteins. The mitochondrial DNA (mtDNA) contains genes for 13 OXPHOS polypeptides, 22 transfer Ribonucleic acid (tRNAs) and two ribosomal RNAs (Papa et al., 2012).

Precedent studies suggest that mitochondria play a vital role in skin physiology. Keratinocyte differentiation is regulated by mitochondria metabolism through the production of mitochondrial reactive oxygen species (ROS). It has been shown that ROS propagate the Notch and b-catenin signals, which promote epidermal differentiation and hair follicle development (Baris et al., 2011). In addition, mitochondria play a role in melanocyte function and pigmentation where prohibitin and complex V were identified as pigmentation modulators (Ni-Komatsu and Orlow, 2007). Moreover, ultraviolet (UV) radiation induces

oxidative stress in the skin which leads to both nuclear and mtDNA damage, with the latter known to be a useful biomarker for both UV radiation exposure and extrinsic skin aging (Birch-Machin and Swalwell, 2010).

Autoimmune diseases of the skin were associated with an increased level of antimitochondrial antibodies (mtABs) such as pemphigus vulgaris. Kalantari-Dehagi et al. proposed that mtABs plays a critical role in pemphigus vulgaris pathology, rather than a bystander (Kalantari-Dehaghi et al., 2013). Altered oxygen respiration, abnormal OXPHOS function and increased ROS productions were displayed when normal keratinocytes were cultured in sera from patients with pemphigus vulgaris. Once treated with mitochondrion-protective drugs such as nicotinamide, minocycline and cyclosporine keratinocytes were protected from the effects of pemphigus vulgaris-derived mtABs (Pope and Thompson, 1999). It was also reported that mitochondrial energy metabolism is alerted in immune cells of Systemic lupus erythematosus (SLE). T lymphocytes of patients with SLE exhibit mitochondrial hyperpolarization increased ROS production and ATP depletion (Perl et al., 2012).

Common mitochondrial single nucleotide polymorphisms (mtSNPs) are categorized into mitochondrial haplogroup clades. These clades reflect the ancestral matrilineal composition of populations. It was shown that mtDNA haplogroups might influence common complex diseases such as melanoma, atopic dermatitis, and psoriasis (Feichtinger et al., 2014a).
In melanoma patients, no difference was found between the frequencies of the major mitochondrial haplogroups between patients and control subjects; however, the frequencies of other SNPs located in the control region of the mtDNA were significantly higher in patients with melanoma compared to controls. In addition, a significant association was found between mtSNPs with mean Breslow thickness and metastasis. Therefore, it was suggested that mtDNA variations could be involved in melanoma etiology and pathogenesis (Ebner et al., 2015). An association between increased level of IgE levels and mitochondrial European haplogroup U in a cohort of children with atopic dermatitis was identified (Raby et al., 2007).
Manipulation of mitochondrial biogenesis is considered in the treatment of a wide range of skin diseases. In accordance, different pharmacological agents that protect mitochondria such as nicotinamide and cyclosporine A have been shown to have a beneficial effect on the progression of pemphigus vulgaris (Kalantari-Dehaghi et al., 2013). Dithranol, a mitochondrial uncoupler that damages mitochondria, has a positive effect in psoriasis (MorliéRe et al., 2006). Glucocorticoids and retinoids used to treat psoriasis all have dose-dependent effects on mitochondrial function (Du et al., 2009). It was shown that

mitochondrial respiration and complex I activity is altered in mononucleate cells from psoriatic patients which are mediated by the up-regulation of GRIM19/STAT3β, which might lead to a chronic activation of T-lymphocytes in the psoriasis pathophysiology (Claudia Piccoli, 2014). It is noted that in pemphigus vulgaris, an increase in mitochondrial activity has a beneficial effect, whereas in psoriasis damaging of mitochondria leads to an improvement in the skin physiology (Feichtinger et al., 2014b).

10. Basement membrane and Psoriasis

Basement membranes (BMs) are cell-associated extracellular matrix (ECM) protein. They cover the basal side of epithelial and endothelial cells. BMs are essential for tissue formation and cell differentiation. They have a tissue-dependent composition, however, laminin, collagens, nidogen, proteoglycans, and agrin are considered the main components (McKee et al., 2007). Laminins are trimeric glycoproteins composed of three different disulfide-linked polypeptides, α, β and γ (Timpl et al., 1979). Update, there are five α, three β and three γ that can form 16 laminin isoforms. Laminin isoforms are named according to their chain compositions. Hence, laminin-411 is composed of α4, β1, and γ1 chains. These isoforms share the cross-shaped structure. They contain a globular laminin N terminal domain and a globular laminin IV domain separated by multiple laminin epidermal-growth-factor-like (LE) domains. In addition, five homologous globular domains (LG domains) are present in the C terminal. LG domains are ligands of cell surface integrin and non-integrin receptor, some can be expressed by the leukocytes (Barlow et al., 1984). Laminin 332, 511 and 521 are known to recognize the a3b1 and a6b4 integrins and laminins 211/221, 332, 411, and 511 have high affinity to thea6b1 integrin (Nishiuchi et al., 2006).

Laminin plays an important role in the formation and the maintenance of the integrity of BMs. they interact with specific cell surface receptors such as integrins this allows endothelial cell anchorage to the BM (Li et al., 2005). When eliminating laminin expression in animal models or the presence mutations in human either it prevents BM formation or can lead to lethal or physiological disorders. For example, laminin α4 null mice during embryonic age known to develop hemorrhage suggesting the important role of this subunit in microvessel integrity (Thyboll et al., 2002). In addition, laminin α5 null mice die late during embryogenesis showing its essential role in the development process (Miner et al., 1998)

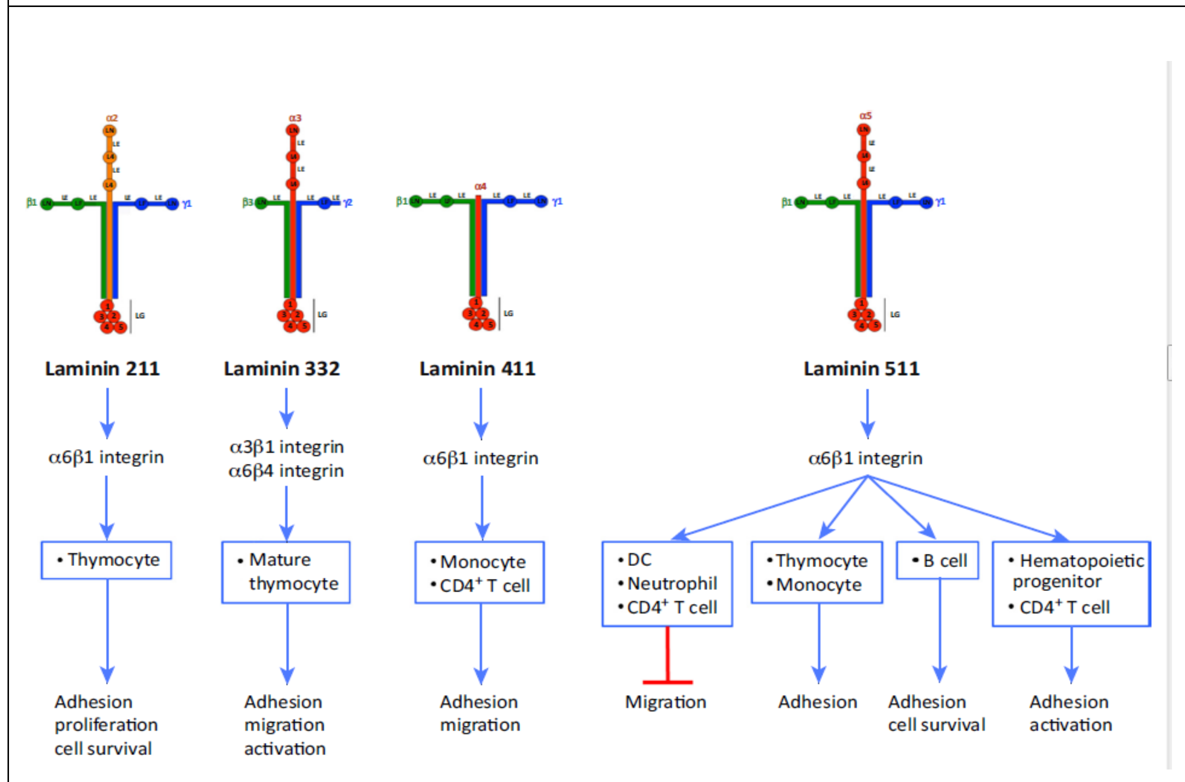
Laminin411 and 511 are essential in bringing T lymphocytes and antigen- presenting cells in the Lymph node cortical ridge (Warren et al., 2014). Also, it is noted that laminins regulate endothendothelial cell function which will influence leukocyte-endothelial interaction (Song et al., 2017).

As shown in Figure 4 specific laminin isoforms influence the migration and function of distinct immune cells via integrin receptors in addition to multiple signaling pathways such as leukocyte proliferation, activation, or survival.

Figure 4: Laminin types and their interaction with immune cells.

Adapted from:(Simon and Bromberg, 2017)

L4, globular laminin 4 domain; LE, laminin epidermal-growth-factor-like; LF, laminin four domain; LG domain, homologous globular domain; LN, globular laminin N-terminal domain



In the context of psoriasis, using immunostaining and confocal laser scanning microscopy a distribution of the basement membrane laminin layer was identified in all samples of psoriatic skin especially in the apex of dermal papillae. In addition to the presence of active keratinocytes (Vaccaro et al., 2002).

Additionally, an essential function of laminin is the stabilization of basal keratinocytes. Its disruption leads to proliferative instability of these keratinocytes accompanied with the expression of proliferative integrins such asa5b1 and extradomain A fibronectin (EDA+Fn) at areas where the basement membrane is discontinuous (Széll et al., 2004)

Laminin is taking considerable attention in immune response and disease presence in psoriasis in later years.

11. Psoriasis research models

In Vitro Models

In vitro models are relatively easy to obtain, cost-effective, and free from ethical and immunological difficulties comparing to animal models. Recent advances in tissue engineering have led to the development of in vitro reconstructed human epidermis models that mimic normal stratified human epidermal morphology (Bracke et al., 2013). The absence of blood vessels and microenvironment are considered as limitations of the in vitro psoriasis skin model nevertheless, this model still useful for investigating pathogenic mechanisms of the disease. Two main types of invite models are described below.

Two-Dimensional (2D) Engineered Skin Psoriatic Cell Model

Using skin cells from freshly isolated lesional psoriatic skins this was the initial attempt to generate an in vitro model of psoriasis. It was reported that the disease phenotype, which was assessed by examining *CAMP*, *DEFB4*, *PI3*, and *TNF* is known to be a marker of psoriasis, were lost during in vitro cell culture expansion (Leigh et al., 1995). To maintain the psoriatic phenotype, specific cytokines were required. Due to the lack of reproducibility, this approach is considered inefficient and does not meet the needs.

Hence, immortalized human keratinocyte cell lines (HaCaT) were used for the study of psoriasis. Compared to virally transformed keratinocyte cell lines, HaCaT is capable of expressing differentiation-specific gene and markers products such as keratins 1 (KRT1) and 10 (KRT10), involucrin and filaggrin (Boukamp et al., 1988).In addition, a broader spectrum of keratins can be expressed such as keratins associated with simple epithelia (e.g., KRT7, KRT8, KRT18, and KRT19). For selection of the best proinflammatory mix different cytokines were used such as IL1α and TNF-α, IL1 isoforms and Oncostatin-M, a potent keratinocyte activator that induces similar effects as TNF-α, IL1α, IL17, and IL22, and regulates many genes related to innate immunity, angiogenesis, adhesion, and motility. The combined effects of all of these selected cytokines appeared to mimic some features of psoriasis(Mezentsev et al., 2013)

Co-culture systems are another method that has long been used to study the interactions between cell populations and to cell-cell interplay research of any kind. It is a cell cultivation set-up, in which different populations of cells are grown with some degree of contact between them. Different studies have been conducted and succeeded to have demonstrated the importance of cross-talk between keratinocyte and lymphocytes or macrophages in modeling the disease in vitro (Schönefuss et al., 2010; Bracke et al., 2013)

Reconstituted Human Epidermal Models (3D)

Commercially available skin equivalents, mostly derived from foreskin keratinocytes, mimic normal skin and are used in a wide range of biological studies. The addition of fibroblasts or

defined growth factors to the equivalents stimulated the development of the epithelium with good morphology. It was described that using reconstructed human epidermis incubated with IL17, IL22, or IFN-γ exhibit features of the psoriatic epidermis (Chiricozzi et al., 2014)

Psoriasis animal models

Animal models have enhanced our understanding of the mechanisms of psoriasis for preclinical treatment. Some of the most important findings are the contribution of the interaction between keratinocytes and immune cells, as well as innate and adaptive immunity, to the pathogenesis of psoriasis. In addition to the exploration of pathogenesis in animal models, drug discovery and development are key applications for utilizing animal models of psoriasis. Different murine models have been developed, these can be divided to the following:

Spontaneous models

The asebia mouse, a spontaneous mutant mouse line that lacks sebaceous glands was reported in 1965 (Gates and Karasek, 1965). Its characteristics are epidermal and dermal thickening, the presence of fibroblasts with abnormal morphology, alterations in collagen and elastin, and increased dermal vascularization and inflammation (Josefowicz and Hardy, 1978). Due to its characteristics, it is considered an epidermal hyperproliferation model used to assess the effects of antiproliferative drugs (Brown et al., 1988). Due to the absence of T cells and neutrophil infiltration, this mouse turns out to be a limited psoriasis model (Brown and Hardy, 1988).

The flaky skin (fsn) mutant mouse described as a mouse model of psoriasis, characterized by gray-white hyperkerotic plaque epidermal hyperplasia, dermal inflammation with leukocytes and macrophages infiltration, and dilated dermal blood vessels. However, this model is unlikely to be a T cell-based immunopathogenesis which lacks mature B and T lymphocytes (Pelsue et al., 1995).

A spontaneous mutation in exon 1 of Sharpin in mice causes progressive multi-organ inflammation, which prominently manifests with chronic eosinophilic and keratinocyte hyperproliferation and severe inflammation in many organs, resulting in the chronic proliferative dermatitis (cpdm) mouse model (HogenEsch et al., 1993). These mice develop systemic inflammation characterized by the accumulation of eosinophils, macrophages, and neutrophils, most prominently in the skin, which are characteristics of psoriasiform dermatitis. Unlike psoriasis, the inflammation in this model develops independently of B and T lymphocytes and is driven by Th2 cytokines such as IL4, IL5, and IL13, and the lesions respond to IL12 treatment (Potter et al., 2014)

Genetically engineered

Genetically engineered or transgenic models are mice with specific gene alterations resulting in the overexpression or loss/knockout (KO) of a particular protein. Some common examples of whole-body KO mice are the $Il1rn^{-/-}$ (Shepherd et al., 2004) and hypomorphic CD18-null mice (Bullard et al., 1996), resulting in the spontaneous development of a psoriatic-like phenotype.

Tissue-specific and conditional transgenic mice have been also developed and these offer many advantages over those with germline alterations. A gene perturbation in a specific cell population or tissue type usually overcomes the lethality commonly seen with traditional germline alterations. Several examples of psoriasis-like tissue-specific mice with overexpression of a particular gene have been described such as the K14-AREG, K14-VEGF, and K5-Stat3C mouse models (Cook et al., 1997; Detmar et al., 1998; Sano et al., 2005). Equally, tissue specific-KO mice have been described, such as the K14-Cre-$Ikk2^{fl/fl}$ mouse model(Stratis et al., 2006).

The tissue-specific model systems also allow further phenotype modifications through limiting gene perturbations to specific cell populations or tissue types that are under the control of inducers or repressors of gene expression. The K5-CreERT2 $JunB^{fl/fl}$ c-$Jun^{fl/fl}$ mouse model(Zenz et al., 2005) is a common example of a tissue-specific inducible KO mouse. In contrast, the K5-IL-17C (Johnston et al., 2013)) and KC-Tie2 (Wolfram et al., 2009) mouse models represent tissue-specific overexpressors, in which the gene of interest can be repressed by administered doxycycline.

Xenotransplantation

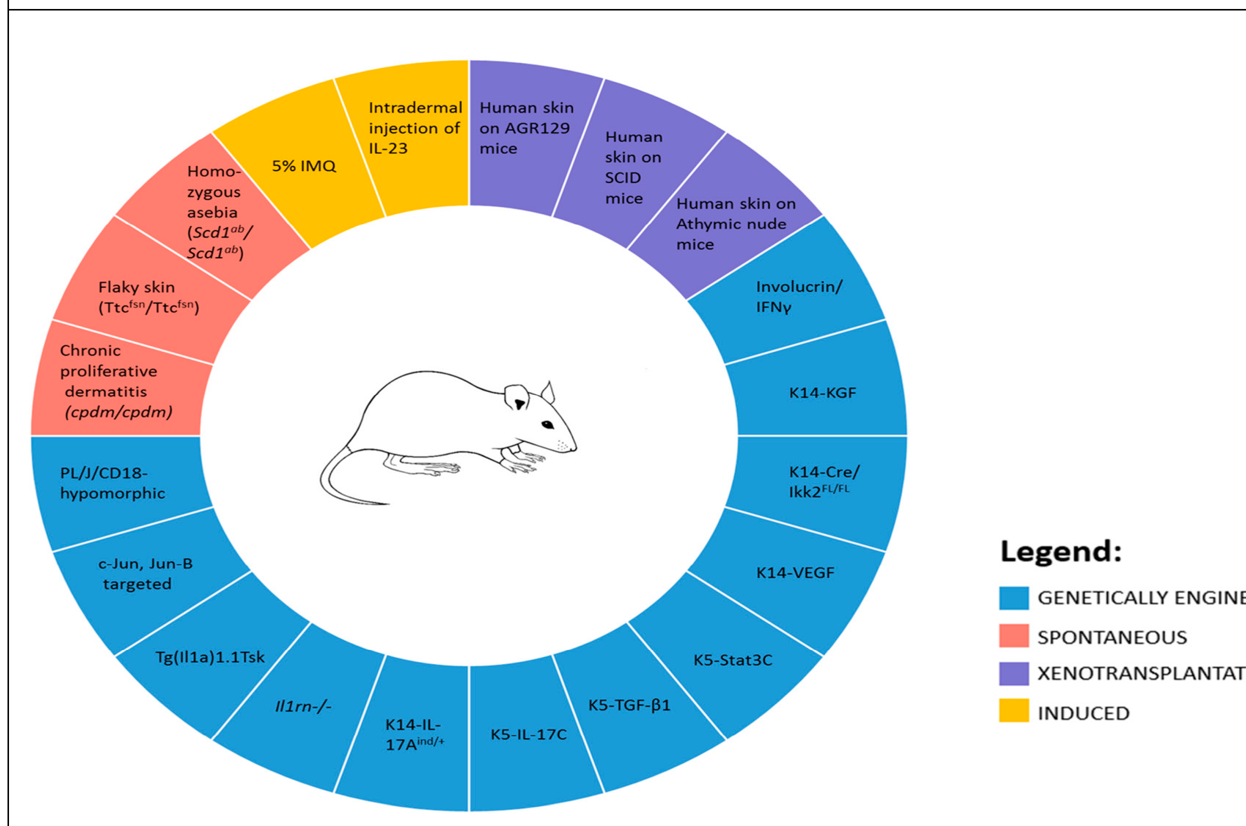
The xenograft or humanized models are created when mice are engrafted with human tissues or cells. In preclinical psoriasis studies, nonlesional or lesional psoriatic skin is transplanted on the backs of immunocompromised mice, such as severe combined immunodeficient or AGR129 mice. Both model systems don’t undergo tissue rejection due to the absence of B and T lymphocytes, while AGR129 mice also lack type I and II IFN receptors and $Rag\text{-}2^{-/-}$, this results in impaired natural killer cell activity (Boyman et al., 2004). In this way, transplanted human tissues develop into psoriatic plaques because of the expansion of resident immune cell populations found in donor skin. The main advantage of using the xenograft models of psoriasis is that they use human-derived tissues and, therefore, most closely mimic the immunologic and genetic basis of the human disease.

Directly induced approaches

The most common example of this model include the repeated application of immune-activating chemicals to the skin of mice, such as imiquimod (van der Fits et al., 2009),12-O-tetradecanoylphorbol-13-acetate, oxazolone, and 2,4-dinitrofluorobenzene (DNFB) (Asherson and Zembala, 1970; Röse et al., 2012; Stanley et al., 1991; van der Fits et al., 2009). Another way to induce psoriasis-like inflammation in the skin by the intradermal injection of proinflammatory cytokines such as IL23 (Kopp et al., 2003)

Figure 5: Model of psoriasis in mouse

Summarize the four models of psoriasis: genetically engineered spontaneous, xenotransplantation and directed induced. Adapted from (Bocheńska et al., 2017)



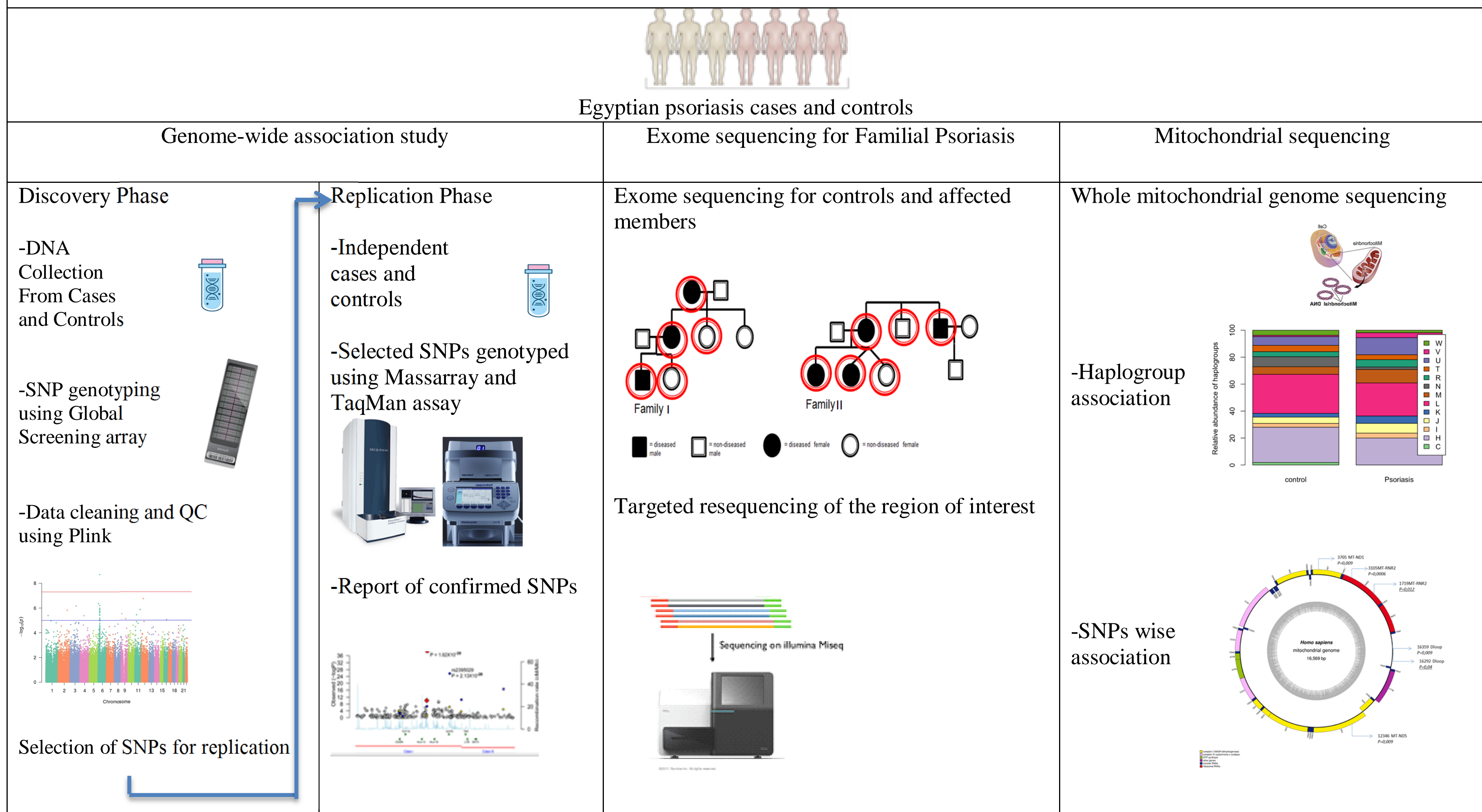
Aim and Objectives

The study investigates for the first time psoriasis in an Egyptian population.
Three different genetic analysis methods were carried out to detect the effect of ethnicity on the genetic background of psoriasis. Below are the three main parts of the study:

- Identification of new psoriasis susceptibility loci in an Egyptian population and investigation the heterogeneity with another studied population through genome-wide association study.
- Identification of causal gene in two unrelated Egyptian Familial psoriasis cases by exome sequencing
- Investigation of the role of mitochondria in psoriasis pathogenesis through whole genome sequencing of the mitochondrial DNA by trying to detect an association between mitochondrial haplogroup and SNPs with psoriasis susceptibility

Figure 6 illustrates the integrated approaches to carry this study

Figure 6: Flow diagram illustrating the integrated approach used to identify the genetics of psoriasis in an Egyptian population



Methods

Genome-Wide Association Study

1. Ethics

Consent was obtained from patients and controls after approving the study protocol by Local Ethical Committee Institutional Research Board (IRB) of Mansoura and Cairo universities. The investigations were conducted in accordance with the Declaration of Helsinki principles.

2. Sample Collection

A total of 1,427 Egyptian samples were collected. Out of these samples, 702 (253 cases and 449 control samples) were processed for the GWAS discovery phase and 725 (321 case and 404 control samples) for the replication phase. All case and control samples were of Egyptian descent recruited from Cairo University and Mansoura University, which is located on the east bank of the Damietta branch of the Nile about 120 km northeast of Cairo. Healthy blood donors recruited as controls were gender and age-matched. The diagnosis of psoriasis was determined on the basis of the ‘Psoriasis Area and Severity Index’ (PASI)-Score (Fredriksson and Pettersson, 1978). Only patients with a moderate to severe PASI and no history of psoriatic arthritis were included in the study.

3. DNA Isolation

DNA was extracted according to the manufacturer’s instructions from peripheral blood samples of psoriatic patients and healthy donors by using the ‘Qiamp DNA Blood Mini Kit’ (QIAGEN, Hilden, Germany) and the ‘Smart blood DNA Midi prep’-Kit (AnalytikJena, Berlin, Germany).

4. SNP genotyping

Infinium® Global Screening Array

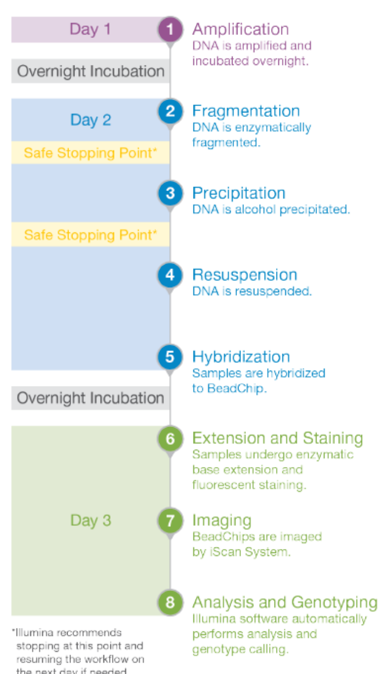
For the discovery phase, approximately 200 ng of genomic DNA was used to type 700,078 SNPs for each sample on the ‘Infinium® Global Screening Array-24v1.0’-Bead Chip on the Illumina platform in collaboration with the Institute for Clinical Molecular Biology, Christian Albrechts University, Kiel, Germany.

The Infinium Global Screening Array 4v1.0 (GSA) Bead Chip is an advanced genotyping array that provides a cost-effective solution for population-scale genetic studies, variant screening, and precision medicine research. The Infinium GSA-24v1.0 BeadChip combines

highly optimized multiethnic genome-wide content, curated clinical research variants and quality control markers for a broad range of clinical research and variant screening applications. It contains a total number of markers 642,824 with a capacity of custom bead types 50 000 markers. Applications include disease association and risk profiling studies, pharmacogenomics research, disease characterization, lifestyle and wellness characterization, and marker discovery in complex disease research. The Infinium HTS format provides a rapid 3-day workflow. It begins with an overnight amplification of the DNA sample. The amplified product is next fragmented by a controlled enzymatic process. After alcohol precipitation and DNA re-suspension, the BeadChip is prepared for hybridization in the capillary flow-through chamber; samples are applied to BeadChips and incubated overnight. The DNA samples are then annealed to locus-specific 50-mers covalently linked to one of over 700,000 beadtypes in an overnight hybridization step. One beadtype corresponds to each allele per SNP locus. After hybridization, allelic specificity is conferred by enzymatic base extension. Products are subsequently fluorescently stained (Figure 7). The intensities of the beads' fluorescence are detected by the iScan system and are in turn analyzed using Illumina software for automated genotype calling. Genotypes were received from the Institute for Clinical Molecular Biology, Christian Albrechts University, Kiel the in standard PLINK analysis formats.

Figure 7: Infinium HTS workflow

Rapid 3- day workflow starting with DNA amplification, then DNA fragmentation and hybridization followed by extension and staining. Adapted from www.illumina.com



Sequenom Mass Array system (iPlex assay)

Out of the 100 top SNPs, 26 SNPs were selected for replication. Genotyping of 16 SNPs was performed using the Sequenom Mass Array system (iPlex assay) at Agena Bioscience, Hamburg, Germany (Table 4). The Sequenom MassARRAY iPLEX^{TM} Assay is a high sample throughput method for genotyping up to ~35 SNPs in a single reaction.

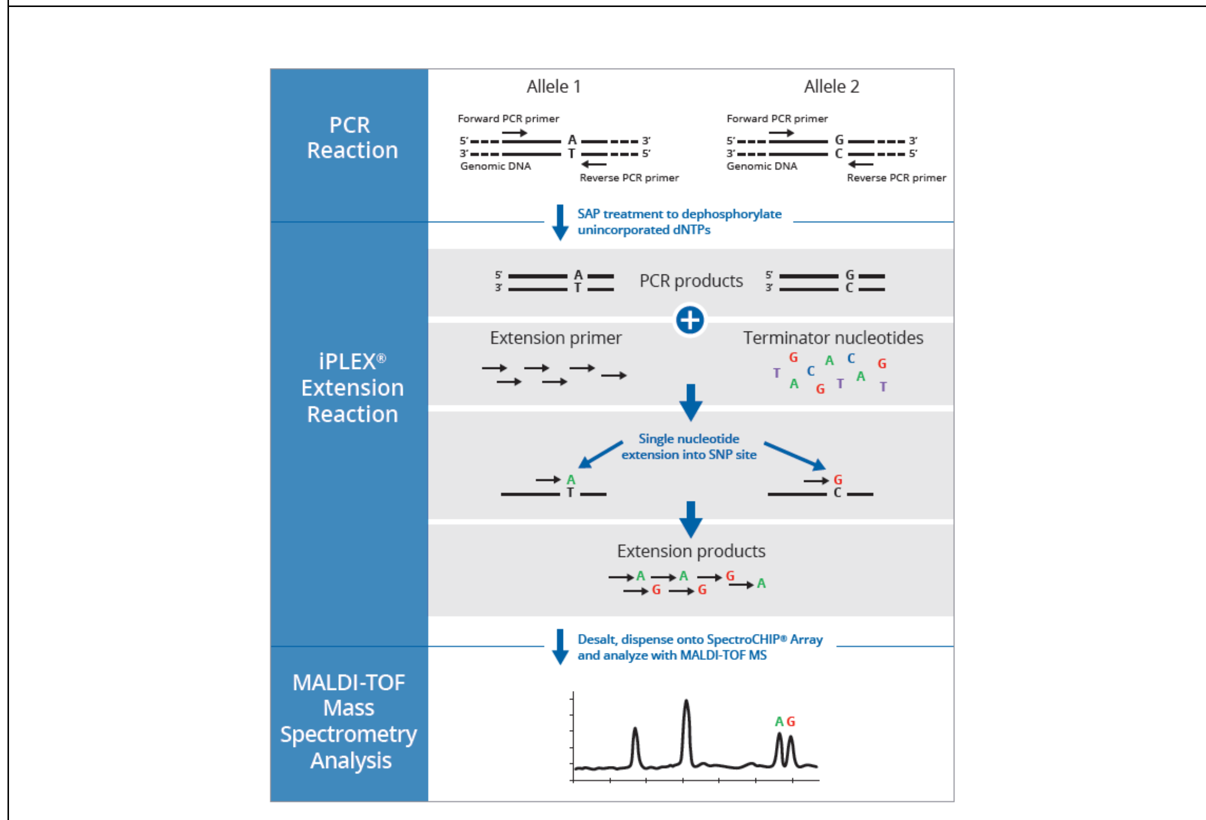
Table 4: SNP ID selected for replication using iPlex assay

	SNP	Chromosome
1	rs12199223	6
2	rs1576	6
3	rs9328377	6
4	rs10960680	9
5	rs4480216	9
6	rs1402216	3
7	rs1484231	3
8	rs55890443	3
9	rs11599750	10
10	rs10832027	11
11	rs12650590	4
12	rs7031901	9
13	rs62255511	3
14	rs7706361	5
15	rs77526264	3
16	rs285982	6

The technique revolves around single-base extension of a primer into the SNP being genotyped, using mass-modified dideoxynucleotide triphosphates bases (ddNTP). The difference in extended primer mass created by the ddNTPs is detected using a matrix-assisted laser desorption/ionization time of flight mass spectrometer (MALDI-TOF MS) and computationally analyzed to produce a genotype (Figure 8). Data genotype results were received as genotyping report from Agena Bioscience, Hamburg.

Figure 8: Sequenom MassARRAY iPLEX^{TM} Assay

Adapted from:" http://www.labclinics.com/en/snp-genotyping-agena/"



TaqMan® SNP Genotyping Assays

Another set of 10 SNPs were replicated using TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA) (Table 5). TaqMan® genotyping with Real-Time Eppendorf Real plex^2 PCR uses fluorescently labeled probes, one for each allele, to bind to amplified DNA during PCR. The fluorescence is then measured at the end of the PCR process as a final end-point read to determine the genotype of the samples. All reagents were purchased from Life Technologies unless otherwise stated. The Allelic Discrimination Plot of sample intensities is then exported to the TaqMan Genotyper Software that applies an algorithm to cluster the samples and assign a genotype. Samples that are high in VIC® dye intensity are homozygotes for allele 1, samples that are high in FAM^{TM} dye intensity are homozygotes for allele 2 and samples that have roughly equal dye intensities are heterozygotes. Genotype clusters can be manipulated manually by the user (Figure 9). Once all markers have been reviewed, the data is extracted for downstream analysis.

Figure 9: TaqMan probe chemistry mechanism

Adapted from "https://www.sciencedirect.com/science/article/pii/S002751070500031X"

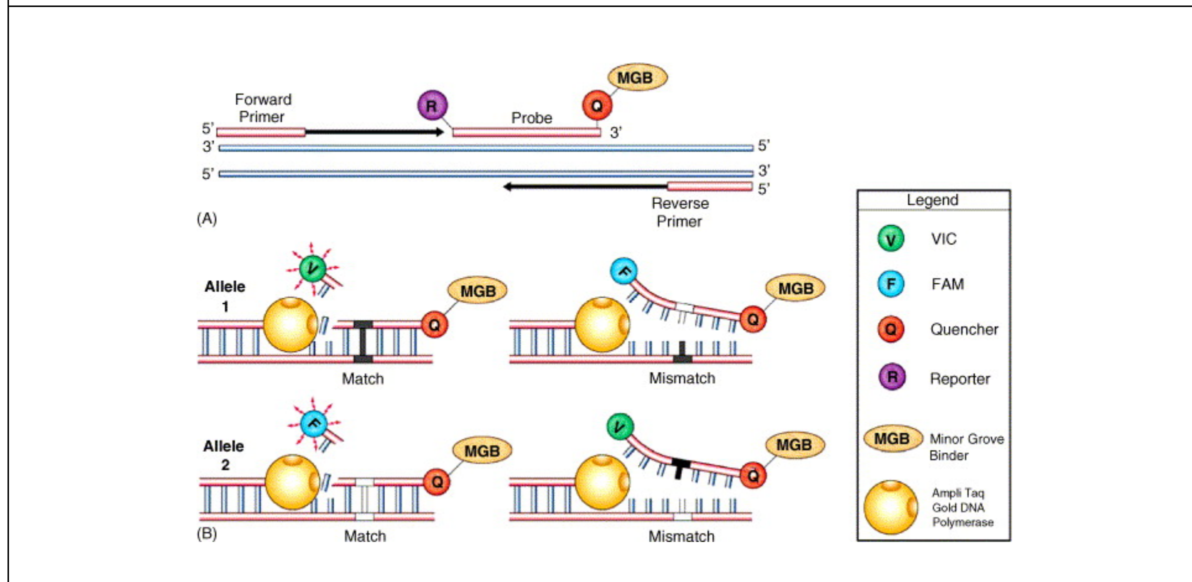


Table 5: SNP ID replicated using TaqMan assay		
	SNP	Chromosome
1	rs1265181	6
2	rs7552136	1
3	rs3770019	2
4	rs1352714	4
5	rs3218634	3
6	rs11170177	12
7	rs16998073	4
8	rs2066845	16
9	rs35322532	2
10	rs16936870	8

5. Association Analysis

Quality Control

5.1.1 Per-individual Quality Control

Exclusion of outlying missing genotype and heterozygosity rates

Variation in DNA sample quality can have substantial effects on genotype call rate and genotype accuracy. Samples of low DNA quality or concentration have low call rates and

genotype accuracy (Laurie et al., 2010). Individuals with more than 10% missing genotypes and increased heterozygosity were excluded (Anderson et al., 2010). The genotype failure rate and heterozygosity rate per individual are both measures of DNA sample quality. Autosomal heterozygosity is the proportion of heterozygous genotypes across an individual's genome, excluding the sex chromosomes, compared to the total number of non-missing SNPs genotyped. In datasets containing a large number of SNPs, autosomal heterozygosity is calculated in order to identify contaminated or population stratified samples as indicated by high heterozygosity or samples that are subject to inbreeding or genotype error as indicated by low heterozygosity. In this study, autosomal heterozygosity was calculated in PLINK using the "--het" command (Purcell et al., 2007).

Mean heterozygosity across all individuals was inspected to identify individuals with an excessive or reduced proportion of heterozygote genotypes given by the below equation:

$$\left[\frac{\text{Number of nonmissing genotypes} - \text{Observed number of homozygous genotypes}}{\text{Number of nonmissing genotypes}}\right]$$

A plot was produced of sample genotype missingness rate against heterozygosity.

Identification of duplicated or related individuals

The presence of duplicated or related first- or second-degree relative's samples creates bias in the association analysis as certain genotypes will become over-represented in the dataset producing false-positive results (Patterson et al., 2006).

To detect duplicate and related individuals we calculated a metric identity by state(IBS) for each pair of individuals and we identified based on the average proportion of alleles shared in common at genotyped SNPs identify pairs of individuals that share more alleles than would be expected by chance.

The identity by descent (IBD) which is the degree of recent shared ancestry for a pair of individuals can be estimated with genome-wide IBS, giving that IBD = 1 for duplicates or monozygotic twins, IBD = 0.5 for first-degree relatives, IBD = 0.25 for second-degree relatives and IBD = 0.125 for third-degree relatives. One individual of the pair of samples with an IBD >0.1875 was excluded. This value was chosen owing to genotyping error, Linkage disequilibrium and population structure, often creates variation around these theoretical values.

Identification of individuals of divergent ancestry

Principal component analysis (PCA) is the most common method for identifying individuals with large-scale differences in ancestry. PCA is a multivariate statistical method that produces several uncorrelated variables, called principal components, from a data matrix containing

observations across a number of potentially correlated variables. The principal component is calculated so that the first principal component explains as much variation as possible in the data this is followed by the second component and so on. In the PCA model of ancestry detection, the observations are the individuals and the potentially correlated variables are the markers (Price et al., 2006).

In our dataset, a PCA model was built on a subset of 50,836 post-quality control SNPs selected to minimize the contribution from regions of extensive strong linkage disequilibrium and to ensure that only genome-wide effects were detected. First, all samples were analyzed together with the HapMap Phase III (HapMap3) data from three ethnic populations: 113 Yoruba in Ibadan, Nigeria (YRI), 170 Asian (86 Japanese in Tokyo, Japan (JPT), 84 Han Chinese in Beijing, China (CHB) and 112 CEPH (Utah residents with ancestry from northern and western Europe (CEU). Second, we performed PCA on the Egyptian population alone and excluded samples that did not pool with the majority of the subjects (Purcell et al., 2007).

5.1.2 Per-marker Quality Control

The removal of suboptimal markers is a key to the success of a GWAS because they can present as false positives and reduce the ability to identify true associations correlated with disease risk. However, the criteria used to filter out low-quality markers differ from study to study. Great care must be taken to remove only poorly characterized markers because every excluded marker can be a potentially missed disease variant.

<u>SNPs with an excessive missing genotype</u>

Only poorly characterized markers with a call rate less than 90% were removed from further study because every removed marker is potentially a missed disease variant (Anderson et al., 2010).

<u>Hardy-Weinberg equilibrium (HWE)</u>

HWE is an ideal state where all alleles and genotypes at a particular genetic locus remain constant in a population over time. If there is a genetic locus with two alleles A and a, whose frequencies are represented by the letters p and q respectively, then $p + q = 1$. In a diploid population, the genotype of AA is p2 and the genotype of aa is q2, with Aa being 2pq. HWE is therefore calculated using the equation $p2 + q2 + 2pq = 1$. This remains true as long as certain factors are present in the population; there must be random mating, no selection pressures either for or against an allele, no mutations creating new alleles, an infinite population size, no migration or emigration of people between populations and structured

generations with no overlap between parents and offspring. The observed genotype frequencies obtained from the sample are compared with the expected genotype frequencies calculated using the Hardy-Weinberg equation. The difference is converted into a Pearson chi-squared (χ^2) statistic and a P-value is calculated (Wittke-Thompson et al., 2005).

In practice, deviation from HWE expectations is most commonly caused by genotyping error. Since a lot of statistical tests assume HWE, failure to exclude SNPs that deviate from it will increase the false-positive rate. However, deviation from HWE in cases alone is indicative of a genuine association. For this reason, HWE is carried out in controls only. In our dataset, we excluded markers with an HWE P-value<0.00001.

<u>Removal of low minor allele frequency (MAF)</u>
Markers are subject to a minimum MAF threshold, as it becomes more difficult to accurately genotype SNPs with smaller MAF. This is because SNPs with low MAFs have low genotype counts which make sample clustering difficult. Even the genotype is true it is difficult for studies to have enough statistical power to detect a true association at these loci. In our dataset, we excluded markers with MAF< 5% (Wellcome Trust Case Control Consortium, 2007).

6. Statistical analyses

Discovery Phase GWAS Analysis

Association analysis by comparing allele or genotype frequency between the cases and the controls is fundamental to GWAS. SNPs and samples of the genome-wide scan that passed quality control were analyzed using PLINK v1.90 beta (Purcell et al., 2007) and R version 3.4.3 ("R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (URL https://www.R-project.org/). PLINK is an open-source C/C++ which can manipulate and analyze large data sets of genotyped markers for thousands of individuals. It also provides five main functions: data management, summary statistics, population stratification, association analysis, and identity-by-descent estimation. R studio was used to generate graphs using 'qqman' package (Turner, 2014). Another comparable statistical test is the Efficient Mixed-Model Association eXpedited (Emmax). It was used to carry the association analysis. Emmax is a command line statistical test that accounts for the structure of the sample by creating marker-based Kinship Matrix using Balding-Nichols matrix (BN) instead of Identity by descent matrix (IBD) because it is claimed that it is more robust to construct the empirical kinship matrix. (Kang et al., 2010).

In our data, we used the additive logistic regression model with case/control status. We assumed that the change in odds of case status due to each copy of the allele was multiplicative. Odd ratio OR and 95% confidence intervals (CI) are also produced from each test. ORs are a measure of the effect size of each SNP and it is defined as a ratio of the odds of getting the disease in cases compared to controls (Table 6)

Table 6: Odd ratio calculation		
	Cases	Controls
T	A	B
C	C	D
$OR(\mathrm{T}) = \frac{\mathrm{A/B}}{\mathrm{C/D}} = \frac{\mathrm{AxD}}{\mathrm{CXB}}$		

An OR greater than 1 indicates an increased chance of developing disease with a particular variant called a causative variant. An OR less than 1 mean a decreased chance of developing the disease called a protective variant. An OR of 1 indicates no increase or decrease in the odds of getting the disease. The CI is a measure of the reliability of the calculated OR. It is a range based on the observed data that is estimated to contain the true value of the OR. It is expressed in terms of a confidence level which defines the probability the CI has of including the true value of the OR. Thus, a 95% CI would expect to contain the true OR value 95% of the time the test was conducted (Edwards, 1963).

Genomic control was calculated using ‘adjust’ function in Plink software. Genomic control detects and compensates for the presence of fine-scale or within-population stratification during association testing. This is checked by the statistic test that calculates the inflation factor λ, which is estimated from a set of selected markers by comparing the median of their observed test statistics with the median of their expected test statistics under an assumption of no population stratification. f $\lambda > 1$, then population stratification is assumed to exist (Devlin and Roeder, 1999).

HLA imputation

To identify the HLA subtype of the rs12199223, HLA Imputation using attribute BAGging (HIBAG) applying a multi-ethnic model based on the Affymetrix Axiom® UK Biobank Array was applied, this work was done in collaboration with Ms. Mareika Wendorff from Institute of Clinical Molecular Biology (IKMB), Kiel University (Zheng et al., 2014). HIBAG

is a software package for imputing HLA types using SNP data, and it uses the R statistical programming language. It combines the concepts of attribute bagging, with haplotype inference for SNPs and HLA types. Attribute bagging improves the accuracy and stability of classifier ensembles deduced using bootstrap aggregating and random variable selection.

Pathway Analysis

Top SNPs were prioritized at the gene, pathway and network level using the R/Bioconductor package 'Priority index' (Pi), (http://pi314.r-forge.r-project.org). Pi is a genomic-led target prioritization system that focuses on leveraging human genetic data to prioritized potential drug targets at the gene, pathway and network level. Also, it used to enhance early-stage target validation of GWAS associated SNPs. The Pi-package was used to define a set of associated variants at a threshold of $P<5\times10^{-5}$and to generate evidence to support identification of the specific modulated genes that are responsible for the genetic association signal by utilizing knowledge of linkage disequilibrium, 50kb distance of associated variants from the gene and evidence of independent genetic association with gene expression in immune cell types and states. Genes with the highest priority were further used to prioritize pathways that were significantly enriched with top 100 assigned genes (Figure 10).

SNP selecting on for replication

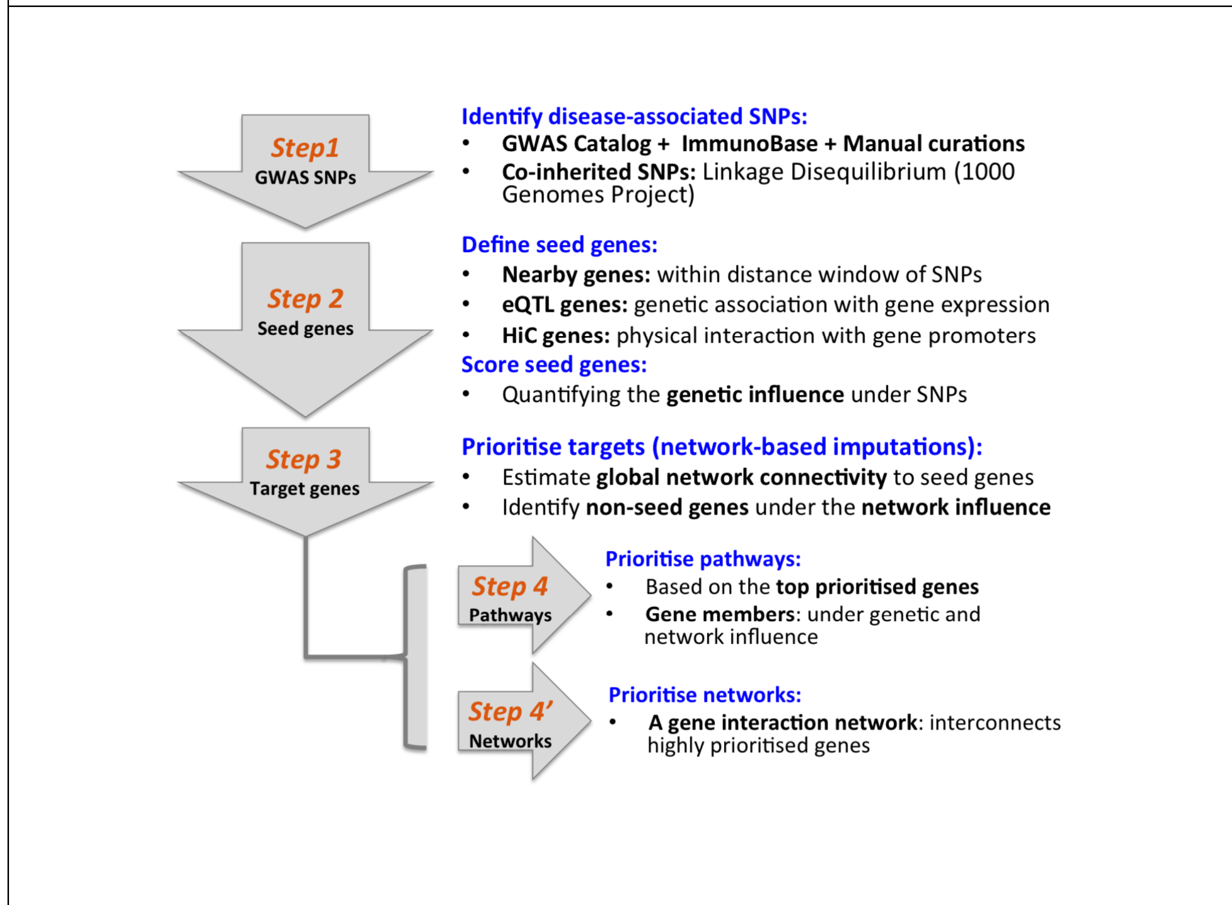
Replication helps to ensure that the genotype-phenotype associated SNPs observed in a GWA phase I study represents a credible association and is not an artifact due to uncontrolled bias. Reproducibility has long been considered a key part of the scientific method.

In our data set, we Replicated 26 selected SNPs.Cluster plots of SNPs showing putative associations were inspected manually using Genome Studio 2.0. Genome Studio 2.0 Visualize and analyze data generated on Illumina array platforms.

Genome Studio 2.0 Visualize and analyze data generated on Illumina array platforms. The graphical display of genotypes in GenomeStudio is a Genoplot, with data points color coded for the call (red = AA, purple = AB, blue = BB). Genotypes are called for each sample (dot) by their signal intensity (norm R) and Allele Frequency (Norm Theta) relative to canonical cluster positions (dark shading) for a given SNP marker. SNPs that did not pass the visual inspection were excluded from further analyses.

Figure 10: Priority Index R package steps applied for pathway analysis

Adapted from Pi User Manual (R/Bioconductor package)



Criteria for selection included the strength of the discovery P-value, a possible biological role of a gene harboring a SNP with some evidence for pathway enrichment, or localization of SNPs with moderate evidence for association to a known psoriasis susceptibility locus.

GWAS SNP replication Analysis

In the replication studies, the 26 SNPs were analyzed using the chi-square $\chi 2$-test (Cochran, 1952). P-values below 0.05 were considered significant. $\chi 2$-test tests the null hypothesis that there is no association between the rows and columns of a contingency table

Using SNSNiPA web-based software we then plotted the SNPs showing association using Linkage disequilibrium to check where they fall in the genome and adjacent to which genes (Arnold et al., 2015).

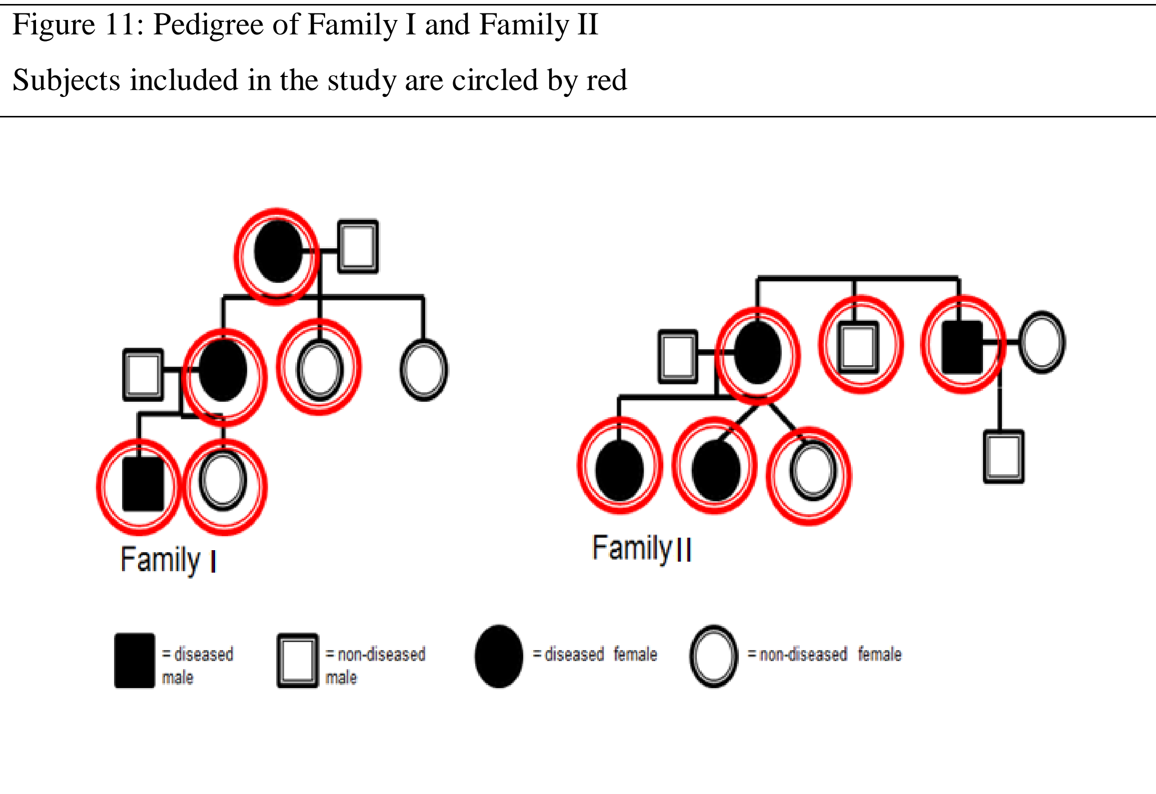
Familial Psoriasis

1. Subjects

Two north unrelated Egyptian families with PsV were recruited from Cairo University. In total 11 samples from both families were collected. From family I, we collected DNA-samples of 3 diseased and 2 non-diseased family-members. From family II, we collected DNA-samples of 4 diseased and 2 non-diseased family-members (Figure 11). Furthermore, additional 524 Egyptian PsV-patients and 808 age-, sex- and population-matched controls were collected from both Mansoura University and Cairo University. The diagnosis of psoriasis was determined on the basis of the 'Psoriasis Area and Severity Index' (PASI)-Score (Fredriksson and Pettersson, 1978).

Figure 11: Pedigree of Family I and Family II

Subjects included in the study are circled by red



2. Whole Exome sequencing

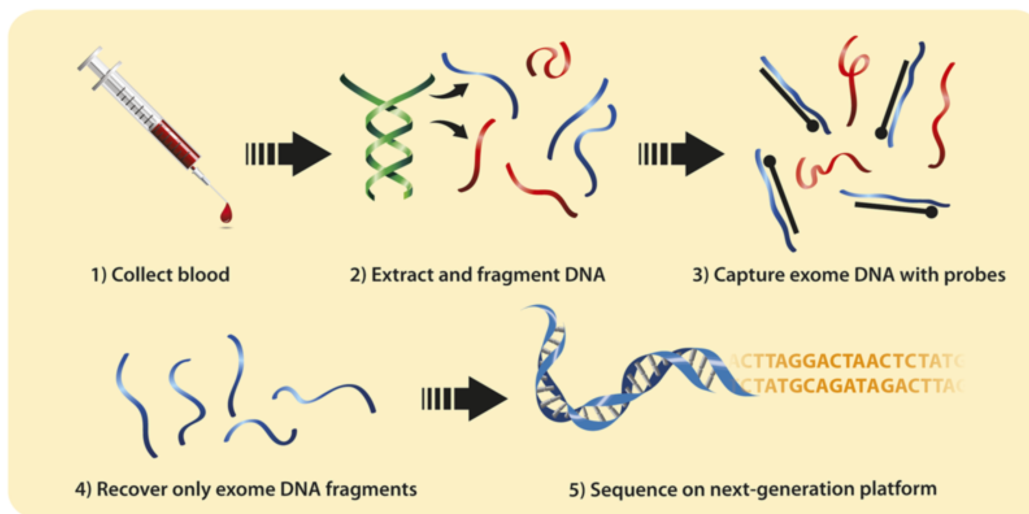
Whole exome sequencing (WES), is a genomic technique for sequencing exomes which are the protein-coding genes in a genome (Ng et al., 2009). WES was performed through a commercial service by Beijing Genome Institute (BGI), Shenzhen, China). Samples were captured using the in solution SureSelect Target Enrichment System (Agilent SureSelect V4 ; Agilent Technologies, Inc., Santa Clara, CA, USA), followed by a paired-end high-throughput

sequencing on reads of 90bp using Illumina HiSeq 2000 (Illumine Inc., San Diego, CA, USA) with a coverage depth of 50x (Figure 12)

Figure 12: Whole exome sequence general protocol

Two main step are the capture of a subset of DNA that encodes proteins then sequence the exonic DNA using any high-throughput DNA sequencing technology

Adapted from (http://epilepsyu.com/blog/quest-diagnostics-to-provide-whole-exome-sequencing-service-to-diagnose-neurological-disorders/)



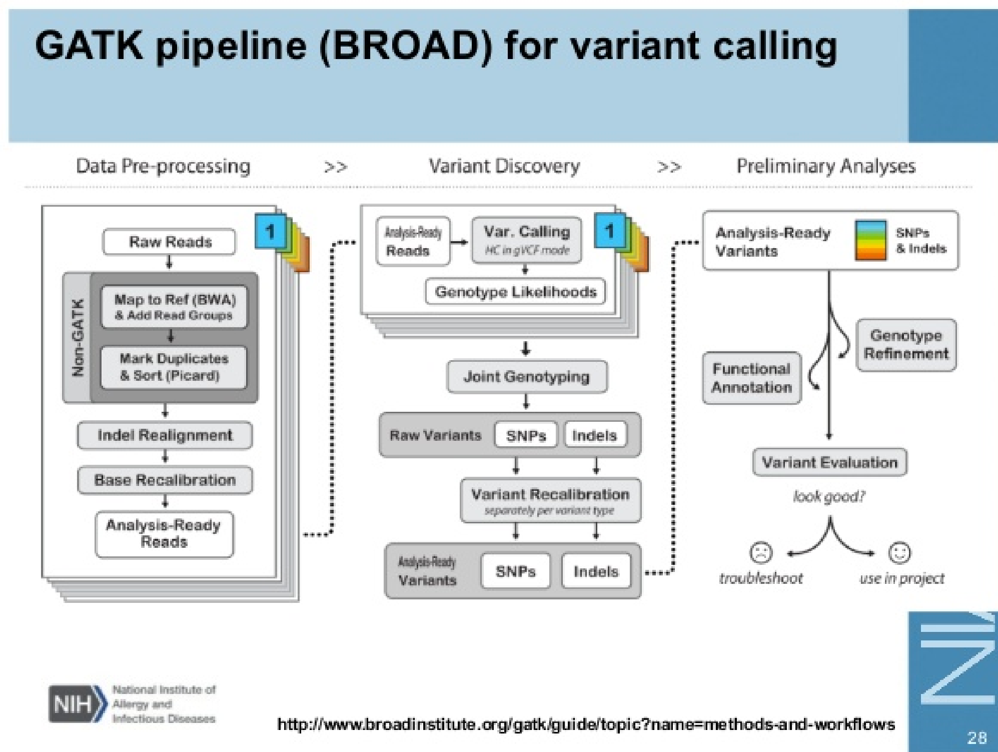
3. Exome sequence analysis and in silico pathogenicity assessment of variants

Exome sequencing was analyzed was done by Dr.Yask Gupta and Dr.Mareika Witte from the Experimental dermatology Lübeck University, using the Genome Analysis Toolkit (GATK) best practice, a structured Java programming framework, used to write efficient and robust analysis tools for next-generation resequencing projects (Figure 13). Read alignment was processed against the human chromosome reference assembly build 38 (GRCh37/hg19) using the Burrows-Wheeler Aligner (BWA) (Li and Durbin, 2009), followed by base quality recalibration with the GATK tool (McKenna et al., 2010). Duplicate reads removed by Picard tools ("http://broadinstitute.github.io/picard.," n.d.). SAM tools were used for calling single nucleotide polymorphisms (SNPs) and insertion and deletions (indels) calling (Li et al., 2009). The sequencing depth and coverage for each individual were calculated based on the alignments.

The annotation of mutations was done by ANNOVAR software which is a software tool that utilizes update information to annotate genetic variants detected. Non-exonic and synonymous variants were excluded. The process was followed by the removal of common variants with MAF >0.05 reported in the single nucleotide polymorphism database 1000 human genome (The 1000 Genomes Project Consortium et al., 2015). To evaluate the pathogenicity of the novel variants, we analyzed the potential impact of a given variant on the function or structure of the encoded protein. Functional consequences of non-synonymous mutations were predicted using the SIFT (Kumar et al., 2009) and PolyPhen-2 (Adzhubei et al., 2010). Among the prioritized variants, exonic mutations predicted to be damaging were considered to be the most promising candidates. Discovered mutations of interest in *LAMA4* were annotated using mutation mapper on the protein domain.

Figure 13: Genome Analysis Toolkit best practice.

(1) Produce analysis-ready BAM files by alignment to a reference genome (2) Variant Discovery by identifying genomic variation in one or more individuals and applying filtering methods appropriate to the experimental design. VCF format is the output file although some classes of variants (3) Filtering and annotation involves using resources of known variation. Adapted from:" https://software.broadinstitute.org/gatk/"

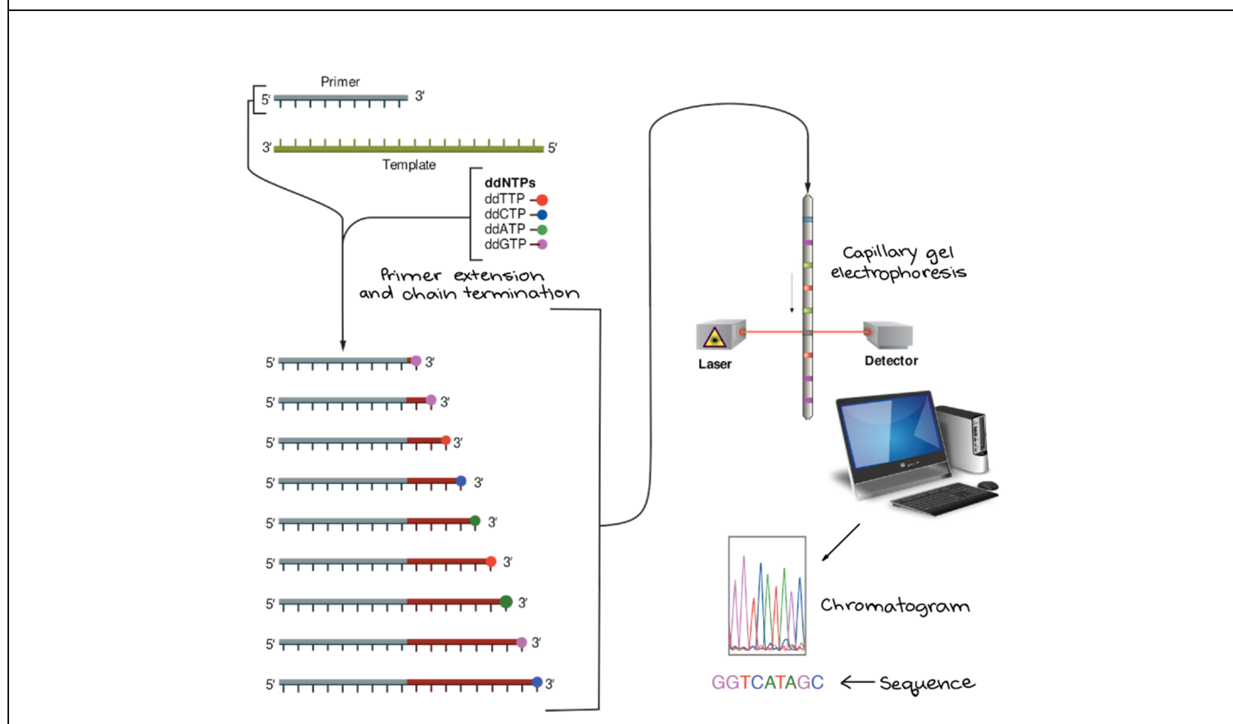


4. Mutation Validation

Sanger sequencing is a method of DNA sequencing based on the selective incorporation of chain terminating dideoxynucleotides by DNA polymerase during in vitro DNA replication (Sanger and Coulson, 1975) (Figure 14). Sanger sequencing on ABI 3130 DNA analyzer was performed in Genewiz global genomics service company to confirm the candidate variants found in LAMA4 from the WES and its segregation within each family member.

Figure 14: Sanger sequencing method

(1) A primer is annealed to a sequence (2) DNA polymerase, dNTPs, and a small amount of all four dideoxynucleotides (ddNTPs) labeled with fluorophores are added to the primer and template. During primer elongation, the random insertion of a ddNTP instead of a dNTP terminates synthesis of the chain because DNA polymerase cannot react with the missing hydroxyl. This produces all possible lengths of chains. (3) The products are separated on a single lane capillary gel, where the resulting bands are read by an imaging system. Adapted from https://www.khanacademy.org/science/high-school-biology/hs-molecular-genetics/hs-biotechnology/a/dna-sequencing



5. Targeted sequencing

The set of exome sequence derived suspected causal mutations in Laminin α4 (LAMA4) gene. We sequenced a cumulative target region of 784,424 bp covering the *LAMA4* and *TRAF3IP2* region at chromosome 6q21 by screening 100 psoriatic and 92 control Egyptian samples. Sequencing was done in the Institute of experimental dermatology using Illumina

Miseq machine (Nr70098435) with the teamwork of Mareike Witte. The region was designed using Illumina Design Studio which is a Web-based tool used to create and optimize custom sequencing probes. Library preparation was performed using Nextera rapid Capture (FC-140-1008) (Figure 15). For the quantification and validation of the genomic library, the Qubit® 2.0 Fluorometer system (Life Technologies) and 2100 Bioanalyzer Instruments (Agilent Technologies) were used. Samples normalization has been performed according to Illumina manufacturer protocol to a final concentration of 10 pM per sample. Pooled amplicon library preparation has been performed according to manufacturer's protocol. Seven sequencing experiments have been carried out, with an average sample number of 14 per run. In order to artificially increase the genetic diversity, 1 % DNA from phage PhiX was added to the library of genomic DNA before loading on the flow-cell.

Runs were performed on Illumina MiSeq sequencer with a V2 flow cell. Reagent cartridges were purchased from Illumina (MS*300 V2 series). Fastq files were generated using Miseq reporter. Data were then analyzed by Dr. Yask Gupta and identified family mutations were checked in the sporadic sequenced samples. Data annotation was done using Annovar (Wang et al., 2010) .

SNP-set (Sequence) Kernel Association Test (SKAT) done by Dr. Yask Gupta was performed using variation within the targeted re-sequenced region to perform a gene-wise analysis. SKAT is a SNP-set level test for association between a set of rare or common variants and dichotomous or quantitative phenotypes (Wu et al., 2011). SKAT collects individual score test statistics of SNPs in a SNP set and computes SNP-set level p-values. This was achieved by applying a window 2.5 KB and spacer of 100 bp.

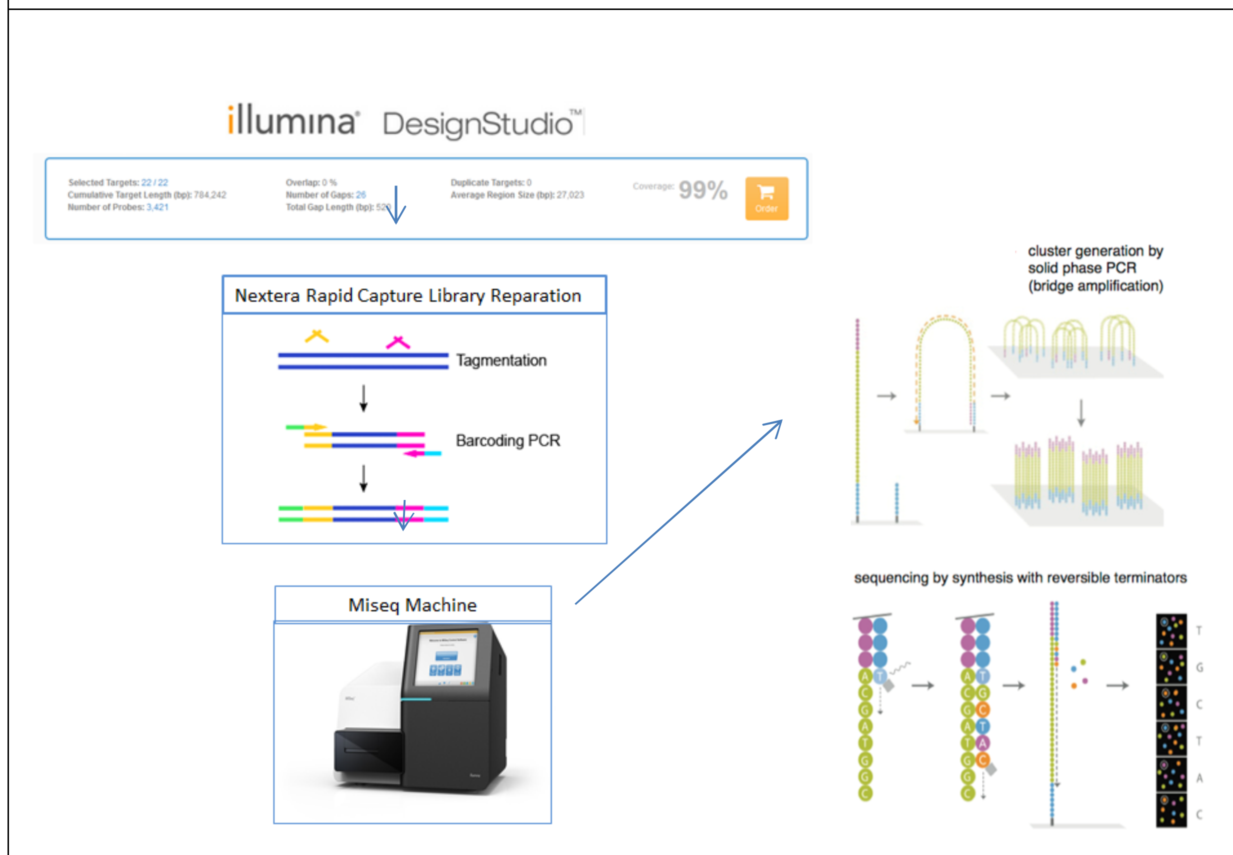
One gene showed a suggestive significance in the SKAT analysis which is Tubulin Epsilon 1 (TUBE1). This was further investigated using co-expression analysis by looking at the genes co-regulated across multiple tissues and experiments. The expression data stems from batch normalization of all Affymetrix human U133 plus 2.0 arrays and Gene expression Omnibus. Person correlation was calculated for all genes. Gene Ontology BP was used as gene sets. In the analysis TUBE1 was excluded and run the gene set enrichment analysis test against the hypothesis that all genes are 0 correlated with the expression of *TUBE1*. This analysis was done by Prof. Hauke Busch.

6. Replication of the SNPs using Taqman

The two SNPs of interest rs13190932 (524cases, 808 controls) rs33980500 (567cases, 501 controls) were successfully genotyped with the fluorogenic 5′-nuclease TaqMan allelic-discrimination assay system (Applied Biosystems, Foster City, CA). The assays were performed under standard conditions on an Eppendorf Reaplplex 2 real-time PCR instrument. Genotypes were analyzed using the χ^2 test (Cochran, 1952).

Figure 15: Targeted resequencing Workflow

(1) Specific sequencing probes designed using Illumina design (2) Library prepared using Nextera Rapid capture (3) Sequencing performed using the Miseq sequencer machine sequencing by synthesis method. Adapted and modified from www.illumina.com



Mitochondrial genome sequencing

1. Subjects

We recruited 110 Psoriatic patients and 107 healthy controls all Egyptian from Mansoura University and Cairo University. Consent was obtained from patients and controls after approving the study protocol by Local Ethical Committee Institutional Research Board (IRB) of Mansoura and Cairo universities. The investigations were conducted in accordance with the Declaration of Helsinki principles. The PASI score was moderate to severe and patients with psoriasis arthritis were excluded.

2. Whole Mitochondria sequencing

Whole genome mitochondria sequencing was carried out using Illumina sequencing technology by the lab technician Ms.Miriam Freitag. mtDNA enrichment from total DNA by PCR was followed by sequencing of the entire mtDNA genome. Two mtDN amplicons (MTL-1and MTL2) were generated by long-range PCR amplification with specific primers to enrich the mitochondrial genome (Table 7). Each amplicon, MTL1: 9065 bp; MTL2: 11170 bp, were run on a gel electrophoresis and purified. Samples are measured using a Qubit plate reader. Then an equal molar concentration of each sample was prepared. Using the Nextera XT DNA Library prep samples were than tagmented, a process where DNA is fragmented and adapter sequenced are added in one step, and a pool of 24 samples was prepared and run on the Miseq sequencer machine using PE Miseq reagent Kit Illumina V2 (Figure 8).

Table 7: Mitochondrial primers sequence

MTL-F1 and MTL-R1 used to generate amplicon MTL-1 and MTL-F2 and MTL-R2 to generate M

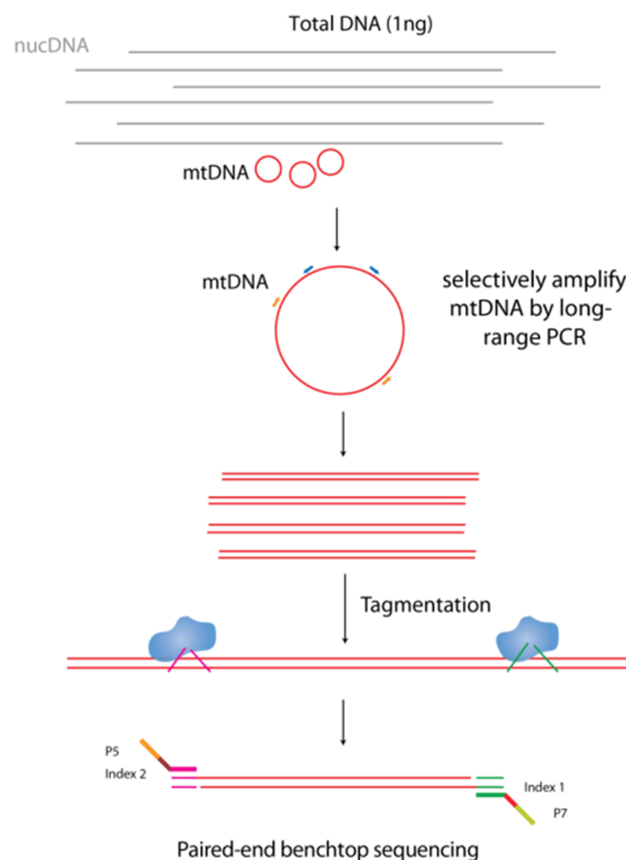
Adapted from:http://emea.support.illumina.com

Primer	Sequence
MTL-F1	5′- AAA GCA CAT ACC AAG GCC AC -3′
MTL-F2	5′- TAT CCG CCA TCC CAT ACA TT -3′
MTL-R1	5′- TTG GCT CTC CTT GCA AAG TT -3′
MTL-R2	5′- AAT GTT GAG CCG TAG ATG CC -3′

Figure 16: Mitochondria sequencing workflow

A total of 1ng genomic DNA is subjected to long-range PCR. Two overlapping mtDNA regions are selected to amplify the mitochondrial genome. Amplicons are than tagmented in a simultaneous process of fragments and ligate sequencing adapters assembly. Libraries are then amplified using specific primers, then sequenced on the Miseq sequencer machine.

Adapted from: https://aging.ouhsc.edu



3. Mitochondrial genome sequence data Analysis

Data analysis was done by Anke Fähnrich from the group of medical systems biology. Fastq files were aligned to reference genome (UCSC.hg19.mtDNA) using the Burrows-Wheeler Alignment tool, duplication was removed and realignment was performed (Li and Durbin, 2009). SNP prediction and Variant allele frequency were conducted using VarScan (Koboldt et al., 2009). VarScan is an open source tool for variant detection, compatible with several short read aligners. In contrast to another variant caller that uses the Bayesian statistics in calling variants which can be confounded by the read depth, contamination or pooled samples,

VarScan claim to apply a robust statistic approach that overpass all the mentioned confounds (Koboldt et al., 2009). MToolBox, a developed pipeline for heteroplasmy annotation and prioritization analysis of human mitochondrial variants, was used for Haplotype prediction was done using (Calabrese et al., 2014). SNP wise analysis was performed applying Fisher exact test on 1158 SNPs (Fisher, 1922).

Results

<u>Genome-Wide Association Study</u>

1. Quality Control Results

In the discovery stage, we conducted a GWAS, screening 253 cases and 449 controls on the 'Infinium® GlobalScreeningArray-24v1.0', including 700,078 variants. After stringent quality control; excluding SNPs with of a call rate <90%, minor allele frequency< 5%, and showing significant deviation from Hardy-Weinberg equilibrium ($P < 10^{-4}$) 312,245 SNPs remained for the association analysis.

As per sample quality controls, we excluded duplicated samples, first- and second-degree relatives, with excessive heterozygosity and samples with a genotyping rate < 90% (Figure 17).

Figure 17: Genotype failure rate versus heterozygosity across all individuals in the study. Dashed lines denote quality control thresholds.

The results of the PCA are displayed in Figure 18. The first two principal components were used to differentiate between the Asian (Chinese and Japanese), African (Yoruba in Ibadan, Nigeria), European population (Northern Europeans from Utah) and the investigated Egyptian

population. as depicted in Figure 18 the Egyptian population clustered with none of these populations, showing that it is genetically distinct.

Figure 18: Evaluation of population stratification in GWAS data using the first two principal component analyses.

Green color represents the African (Yoruba in Ibadan, Nigeria (YRI), purple the Asian (Japanese in Tokyo, Japan (JPT), Han Chinese in Beijing, China (CHB)) and red 112 CEPH (CEU) samples. Black color denotes the Egyptian case and control samples.

PC1: Principle component 1, PC2: Principle component 2

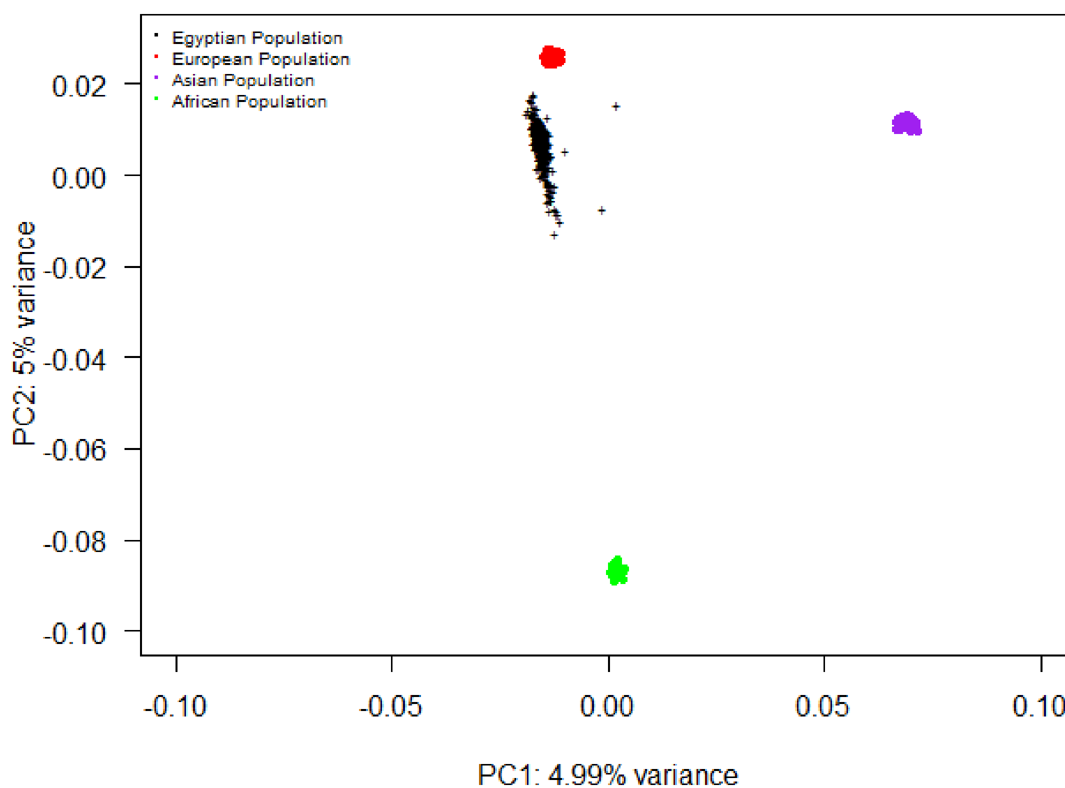
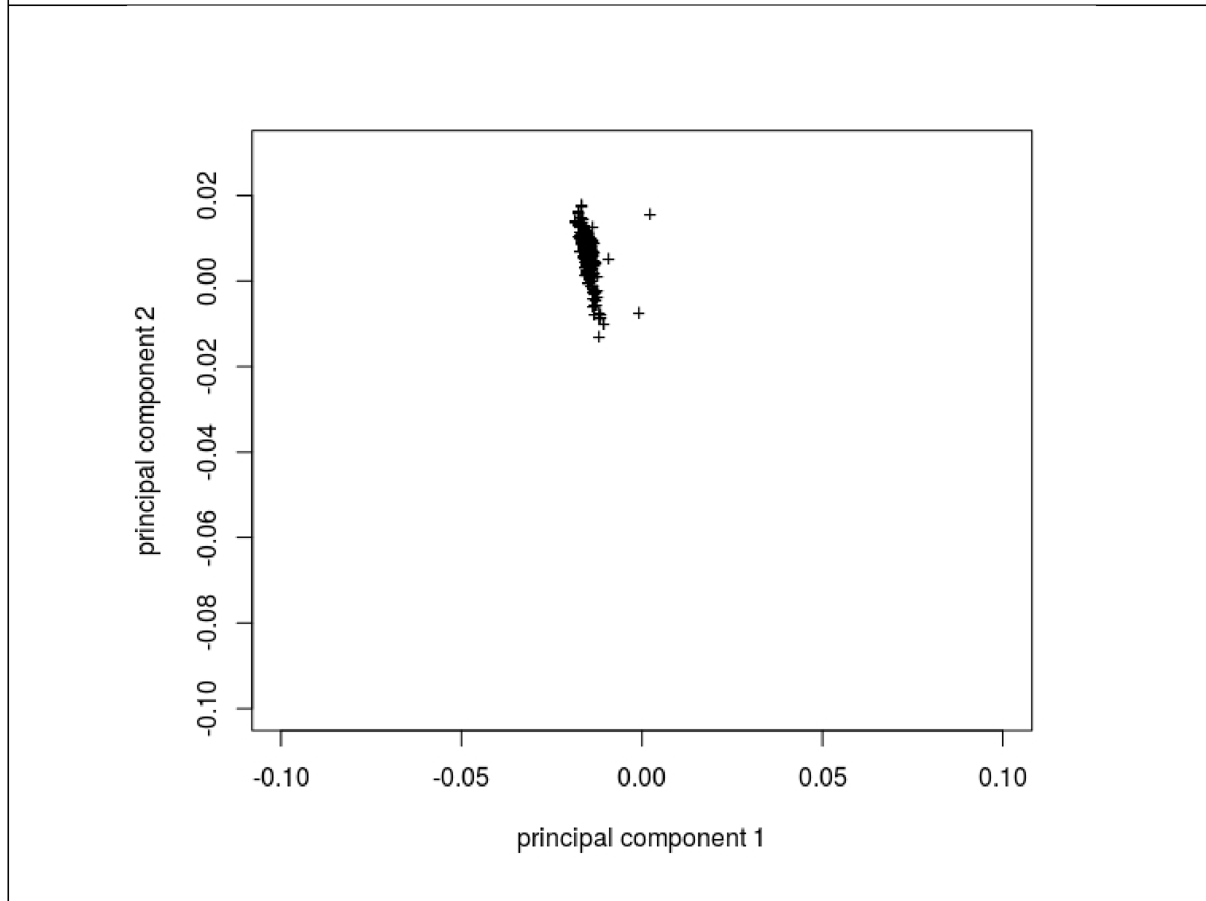


Figure 19 displays the PCA for the cases and controls samples of the Egyptian cases and controls genotyped samples. We excluded the samples lying outside the cluster from further analysis as of potential non-Egyptian origin or introgression of other populations. After this step, 215 cases and 416 controls remained for further analysis.

Figure 19: Evaluation of population stratification within Egyptian cases and controls



2. GWAS Discovery Stage

A logistic regression additive model test was carried out to assess the genotype-phenotype association. The quantile-quantile plot of the observed *P*-values for association (Figure 20) shows a deviation from the null distribution at the tail, likely owing to the strong association observed within the MHC locus as depicted in Figure 21. A genomic control value of λ_{GC}=1.08 indicates the absence of overall inflation of the genome-wide statistical results due to population stratification.

In total, 47 SNPs reached the suggestive genome-wide significance ($P < 5 \times 10^{-5}$). We observed a strong association with the MHC region (Figure 21); the most significant variant within this region was rs12199223 with a *P*-value of 3.55×10^{-09}.

Figure 20: Quantile-quantile (Q-Q) plots of association.
Deviations of the points from the line correspond to loci that deviate from the null hypothesis. The genetic inflation factor lambda is 1.08.

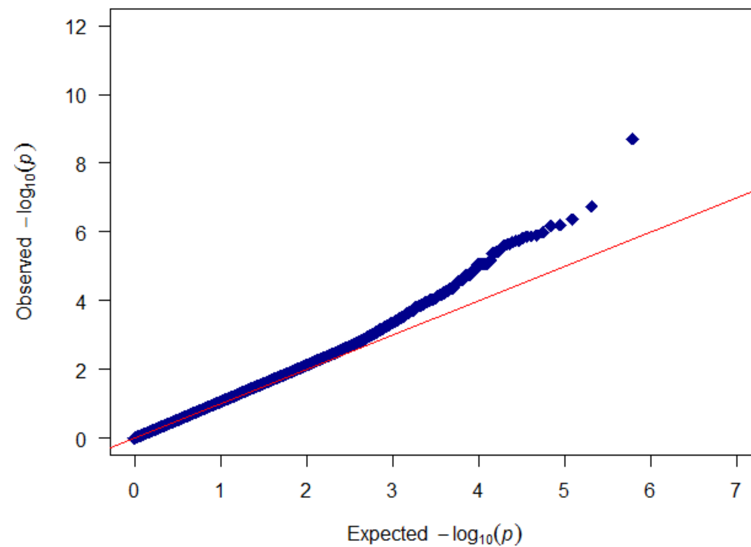
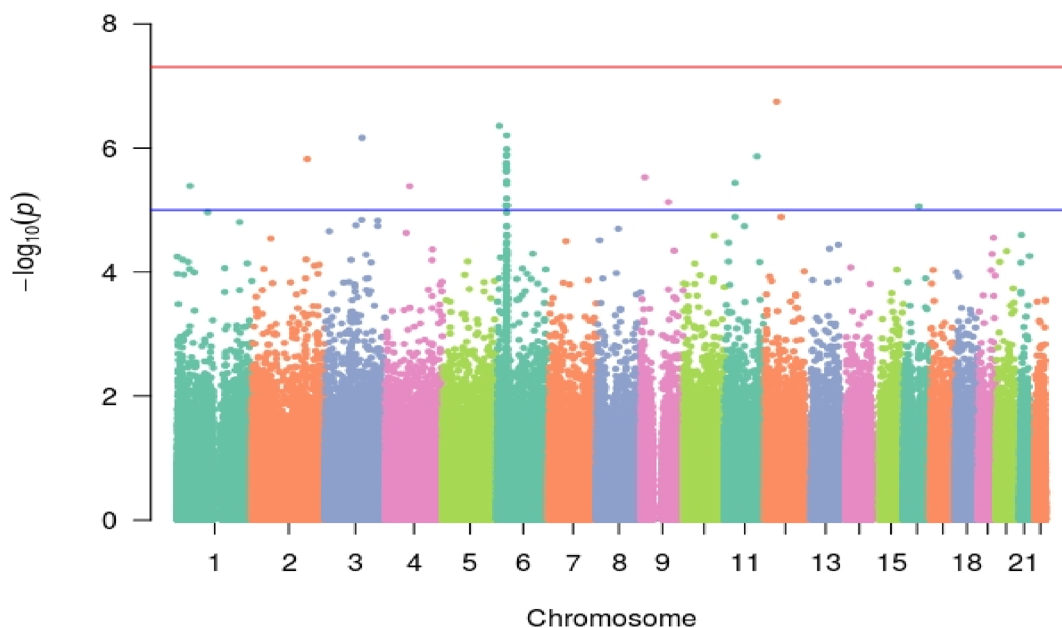


Figure 21: Manhattan plot of the GWAS using the discovery cohort
Comprising 215 cases and 416 healthy controls showing the $-\log_{10}$ *P*-values of the logistic regression test of 312,245 SNPs. The blue line indicates a suggestive association threshold, $P=1\times10^{-5}$ while red line indicates a genome-wide significant threshold $P<5\times10^{-8}$.



Other previously reported psoriasis candidate genes were evaluated for association in our genome-wide results (Table 8); however, we did not find any evidence that supports significant association in the frequently replicated SNPs such as rs9988642 [*IL23R*] *P*=0.07, rs7709212 [*IL12B*] *P*=0.047.

In contrast, TRAF3IP2, a gene that was previously found to be associated with psoriasis in the German population (Ellinghaus et al., 2010b), showed a suggestive evidence of association at the SNP rs76228616 with a *P*-value of $1.29 \times 10^{-4}$and *TNP1* at rs2233278 showed a tendency towards association(*P*=0.003).

Table 8: investigation of published Psoriasis GWAS associated SNPs in the Egyptian population

CHR	SNP	BP	A1	TEST	NMISS	OR	P	Gene
1	rs2201841	67694202	G	ADD	621	0,9557	0,7122	il23R
1	rs4085613	152550018	A	ADD	621	0,9877	0,9221	LCE3D , LCE3A
1	rs1581803	152592281	C	ADD	620	0,9746	0,8415	LCE3A
1	rs12564022	67670755	A	ADD	621	0,9742	0,8308	IL23R
1	rs10794648	24518206	A	ADD	621	0,9643	0,7755	IFNLR1
1	rs2201841	67694202	G	ADD	621	0,9557	0,7122	IL23R
1	rs4649203	24519920	G	ADD	621	1,05	0,694	IL28RA
2	rs3747517	163128824	A	ADD	620	0,9946	0,9664	IFIH1
2	rs842625	61080482	A	ADD	621	0,9714	0,8107	REL
2	rs842625	61080482	A	ADD	621	0,9714	0,8107	REL
2	rs17716942	163260691	G	ADD	621	0,7834	0,1102	IFIH1
2	rs702873	61081542	A	ADD	621	0,9623	0,7509	REL
3	rs7637230	101663555	G	ADD	620	0,5635	$8{,}4E^{-05}$	NFKBIZ
3	rs28512356	189615475	A	ADD	618	0,8264	0,3016	TP63
4	rs6056	155488821	A	ADD	621	0,8228	0,222	FGB
4	rs62324212	123560939	A	ADD	616	1,01	0,9418	IL21
5	rs3213094	158750769	A	ADD	621	0,7301	0,02455	il12B
5	rs2082412	158717789	A	ADD	621	0,7111	0,01383	IL12B
5	rs7709212	158764177	G	ADD	621	0,7774	0,05514	IL12B
5	rs2233278	150467189	C	ADD	621	1,035	0,8727	TNIP1
5	rs17728338	150478318	A	ADD	621	1,005	0,983	TNIP1
5	rs20541	131995964	A	ADD	621	0,9406	0,695	IL13
5	rs20541	131995964	A	ADD	621	0,9406	0,695	IL13
5	rs2853694	158749088	C	ADD	621	1,142	0,282	IL12B
5	rs20541	131995964	A	ADD	621	0,9406	0,695	IL13
6	rs33980500	111913262	A	ADD	620	1,196	0,3711	Traf3ip2

6	rs4406273	31266090	A	ADD	619	2,156	5E-06	HLA-B, HLA-C
6	rs10484554	31274555	A	ADD	621	1,743	6,3E-05	HLA-C
6	rs1265181	31155785	G	ADD	621	1,976	3,9E-05	MHC
6	rs10484554	31274555	A	ADD	621	1,743	6,3E-05	HLA, HLA-C
6	rs12191877	31252925	A	ADD	610	1,704	0,00014	HLA-C
6	rs13191343	31241109	A	ADD	606	1,512	0,00243	HLA-C
6	rs240993	111673714	A	ADD	621	1,248	0,06991	REV3L
6	rs458017	111696091	G	ADD	621	1,07	0,7759	Traf3ip2
6	rs643177	138195693	A	ADD	621	1,297	0,05821	TNFAIP3
6	rs12153855	32074804	G	ADD	621	0,8894	0,502	TNXB, CREBL1
6	rs7769061	111926909	G	ADD	621	0,9762	0,9287	TRAF3IP2
6	rs643177	138195693	A	ADD	621	1,297	0,05821	TNFAIP3
7	rs2533291	157505654	A	ADD	618	1,085	0,6638	PTPRN2
9	rs1076160	135776034	A	ADD	621	1,279	0,04069	CARD9
10	rs7067780	45352419	A	ADD	621	1,05	0,7299	IL2RA
11	rs6590334	128403208	G	ADD	621	0,8502	0,1792	ETS1
11	rs694739	64097233	G	ADD	621	0,9005	0,4495	PRDX5
12	rs2066808	56737973	G	ADD	621	0,7759	0,2576	IL23A
12	rs2066808	56737973	G	ADD	621	0,7759	0,2576	STAT2, IL23A
14	rs12884468	35852486	G	ADD	621	1,396	0,00578	NFKBIA
14	rs8016947	35832666	A	ADD	621	0,8178	0,1107	PSMA6 , NFKBIA
14	rs12586317	35682172	G	ADD	617	0,6876	0,06165	NFKBIA
17	rs4795067	26106675	G	ADD	621	1,027	0,8439	NOS2
17	rs11652075	78178893	A	ADD	620	0,9085	0,4393	CARD14
20	rs909341	62328742	A	ADD	621	0,9203	0,6162	TNFRSF6B
20	rs495337	48522330	A	ADD	621	0,7648	0,07208	SPATA2 , RNF114
21	rs8128234	36470865	A	ADD	621	1,285	0,04799	RUNX1

3. HLA imputation

HLA HIBAG was used to predict the HLA-C genotype represented by the associated SNP rs121992223. Figure 22 represents the quality of the HLA locus prediction, as represented we can see that 80% of the samples have a post probability >80% which reflect the accuracy and sensitively that will affect the HLA-C genotype imputation. The results of HLA-C imputation at rs121992223 position showed that HLA-C 06:02 explains most of the signal from the rs121992223 (Figure 23).

Figure 22: Posterior probability criteria for HLA prediction

Post prob :posterior probabilty

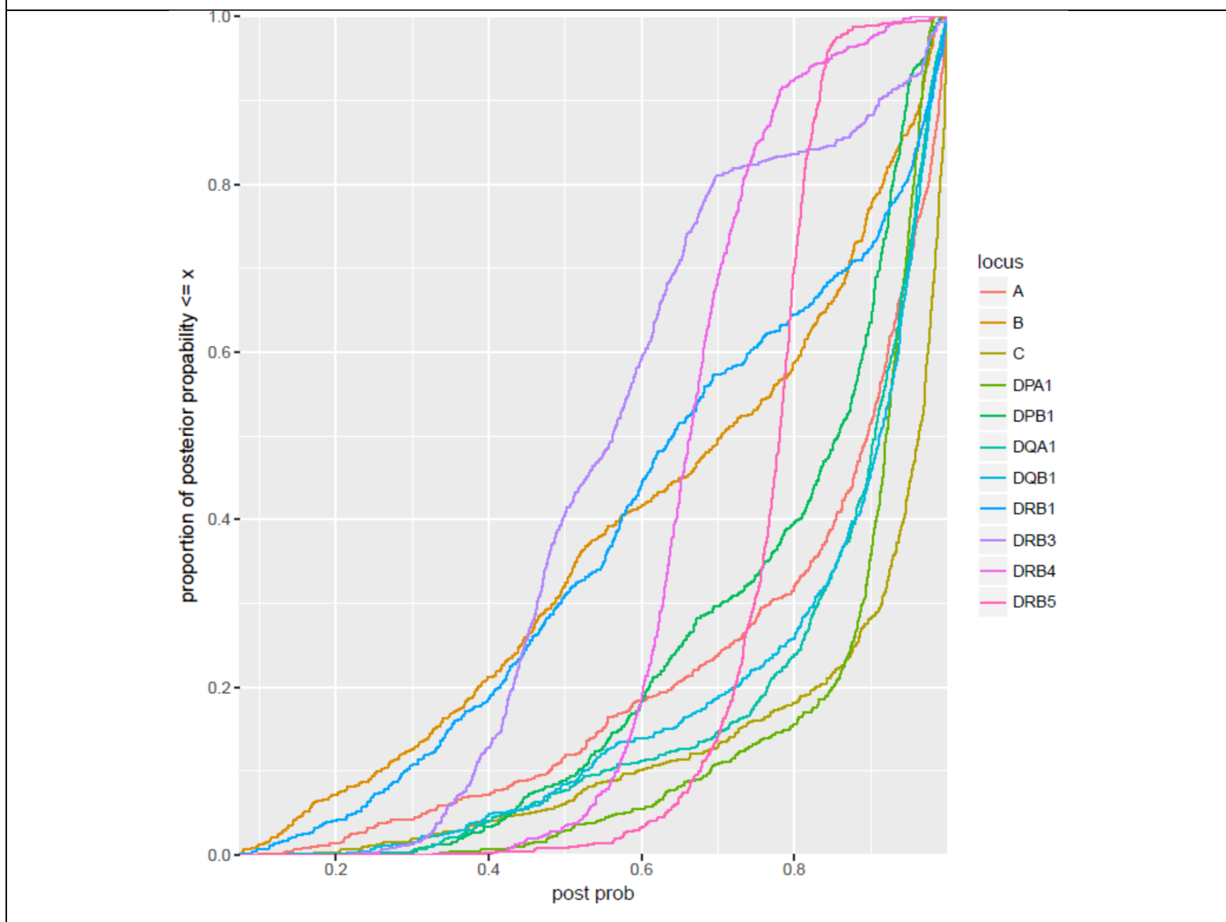
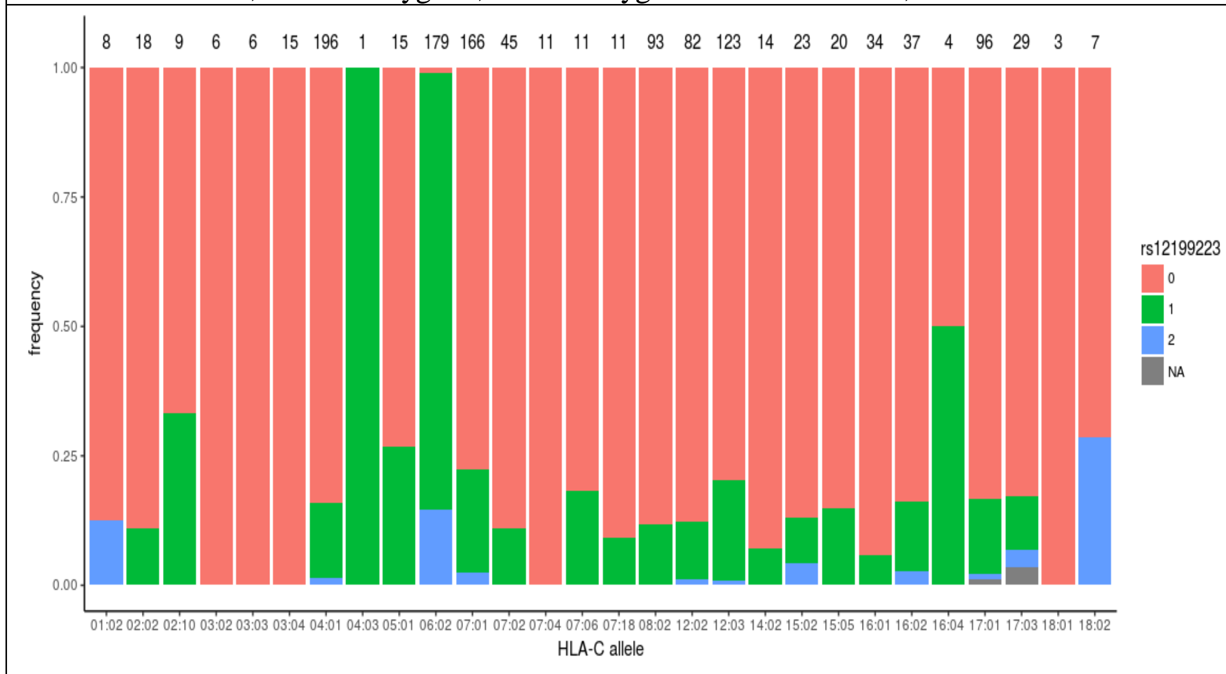


Figure 23: HLA-C allele typing prediction using HIBAG

0: reference allele, 1: Heterozygous, 2: Homozygous for minor allele, NA: not identified

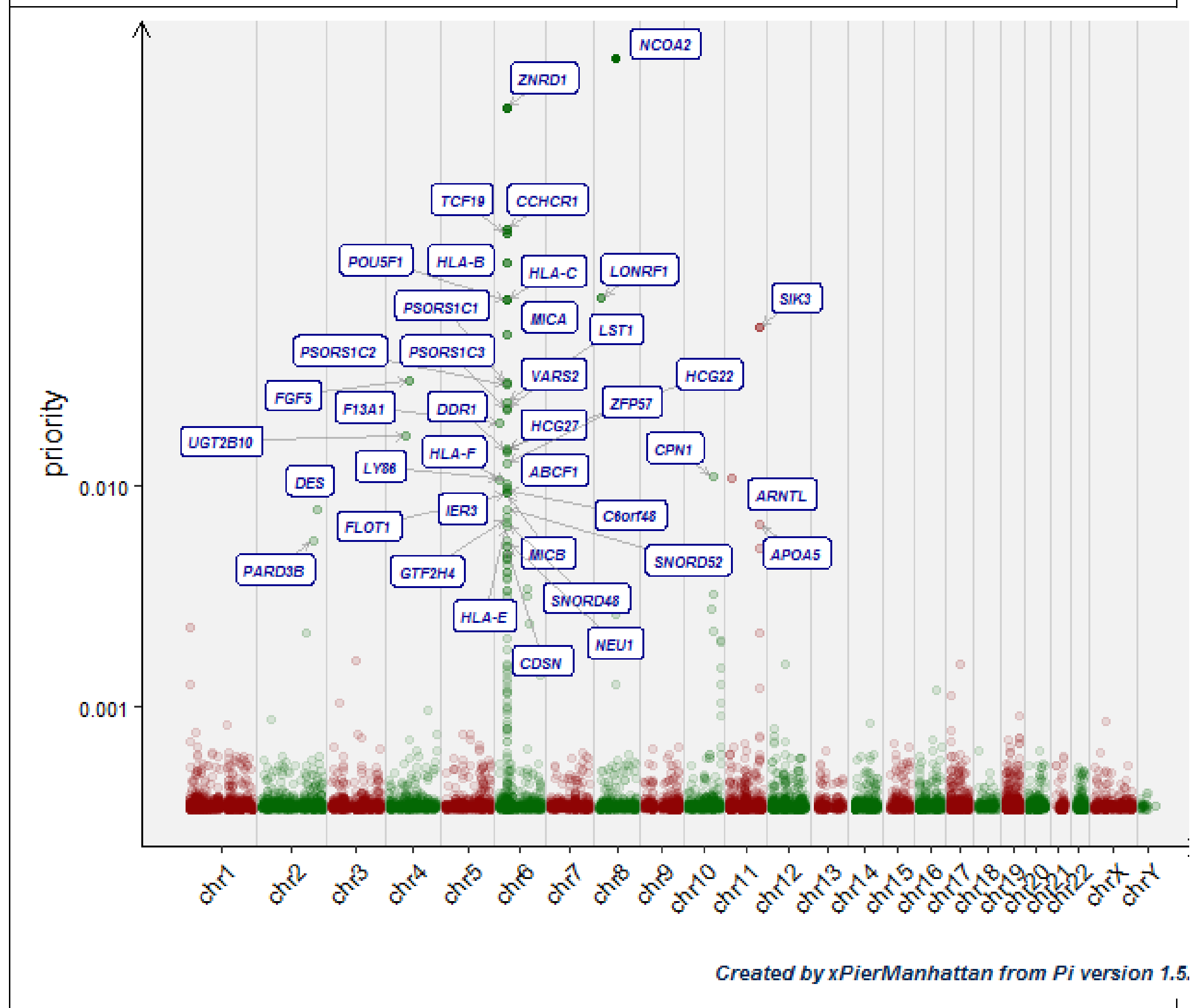


4. Pathway Analysis

Using PI we prioritized the associated SNPs on the gene level and pathway level. Two analyses were conducted one by keeping all SNPs that reached a *P*-value of $5x10^{-5}$ including MHC region and the other excluding SNPs within MHC region. Gene prioritization analysis highlighted genes that were already published to be associated with psoriasis such as HLA-C and CCHCR1 (Figure 24). Analysis of candidate genomic regions captured pathways within the immune system such as antigen processing and presentation, allograph rejection, cell adhesion and natural killer cell cytotoxicity (Figure 25).

Figure 24: Top 40 genes highlighted in Manhattan plot

Priority scores for genes are displayed on the Y-axis along with genomic locations on the X-axis.



After the exclusion of the MHC region, the top enriched gene was *TRAF3IP2*, a gene that was previously associated with psoriasis in the German population (Ellinghaus et al., 2010c) (Figure 26). In addition, new pathways of considerable biological relevance to psoriasis-pathogenesis were enriched such as the 'peroxisome proliferator-activated receptor' (PPARA)-signaling pathway, the 'RAR related Orphan Receptor' (RORA) circadian-pathway and the 'Phosphatidylinositol-4,5-bisphosphate 3-kinase'(PI3K) pathway(Figure 27).

Figure 25: Barplot of prioritized pathways based on top 100 genes including MHC region. Using a compendium of pathways from diverse sources (Canonical, KEGG, BioCarta, and Reactome

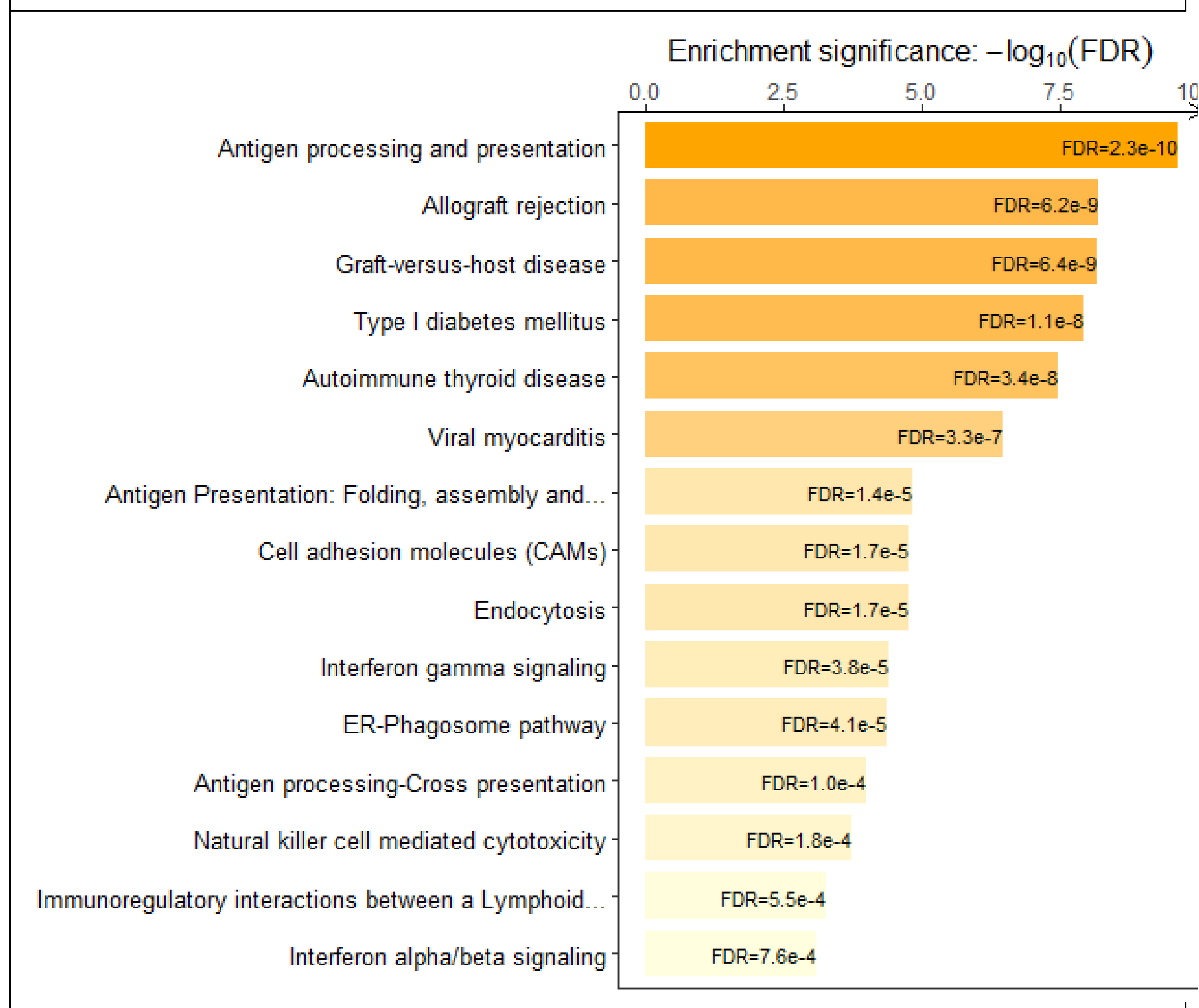


Figure 26: Top 40 genes highlighted in Manhattan plot excluding MHC region

Priority scores for genes are displayed on the Y-axis along with genomic locations on the X-axis.

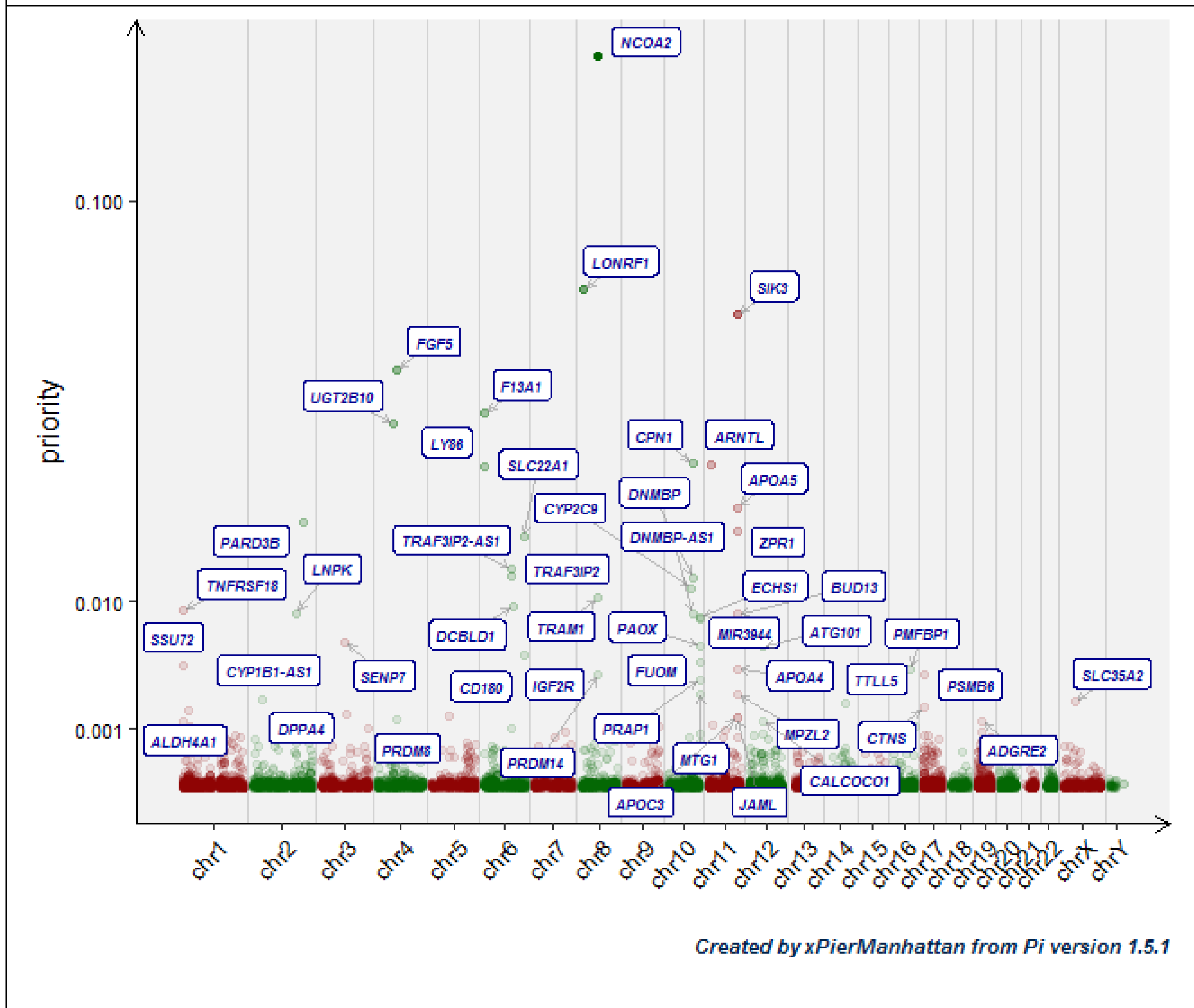


Figure 27: Barplot of prioritized pathways based on top 100 genes excluding MHC region Using a compendium of pathways from diverse sources (Canonical, KEGG, BioCarta, and Reactome

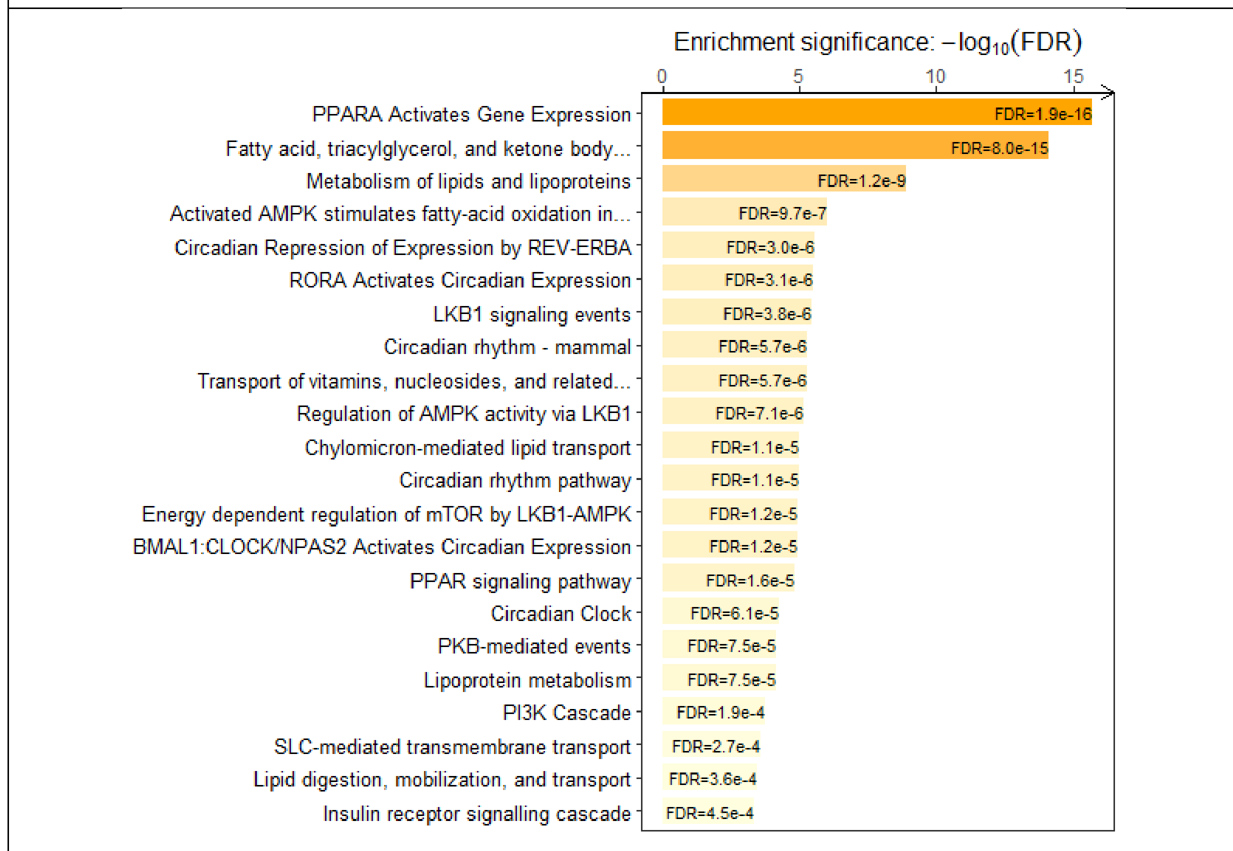


Table 9: Gene Member of the prioritized pathway. The bold gene are associated with psoriasis in our data

Pathway Name	Gene Members
PPARA Activates Gene Expression	ABCB4, ANKRD1, APOA1, **APOA5**, ARNTL, CLOCK, CPT2, FADS1, G0S2, GLIPR1, GRHL1, **NCOA2**, NPAS2, PLIN2, SLC27A1
Fatty acid, triacylglycerol, and ketone body metabolism	ABCB4, ANKRD1, APOA1, **APOA5**, ARNTL, CLOCK, CPT2, ECHS1, FADS1, G0S2, GLIPR1, GRHL1, NCOA2, NPAS2, PLIN2, SLC27A1
Metabolism of lipids and lipoproteins	ABCB4, ANKRD1, APOA1, **APOA5**, APOC3, **ARNTL,** CLOCK, CPT2, ECHS1, FADS1, G0S2, GLIPR1, GRHL1, NCOA2, NPAS2, PLIN2, SLC27A1, SLCO1B3
Activated AMPK stimulates fatty-acid oxidation in muscle	CAB39, CPT2, STRADA, STRADB
Circadian Repression of Expression by REV-ERBA	**ARNTL**, CLOCK, **NCOA2**, NPAS2
RORA Activates Circadian Expression	**ARNTL**, CLOCK, **NCOA2**, NPAS2
LKB1 signaling events	CAB39, CRTC2, **SIK3**, STRADA, STRADB

5. SNP selection for replication

After the exclusion of 9,316 SNPs mapping to the MHC locus (chr6: 25–34 Mb), 35 non–MHC SNPs were found to be associated at the suggestive level. Three SNPs located in the MHC region and 23 SNPs outside the MHC-region were selected for replication according to their *P*-value and contribution to pathways that play a role in psoriasis pathogenesis. SNPs to be replicated were inspected manually using Genome Studio 2.0. Figure 28 illustrates an example of the SNPs cluster criteria necessary to carry it over for replication.

Figure 28: Genoplot for rs12199223 using Gnome Studio 2.0

(red = AA, purple = AB, blue = BB).

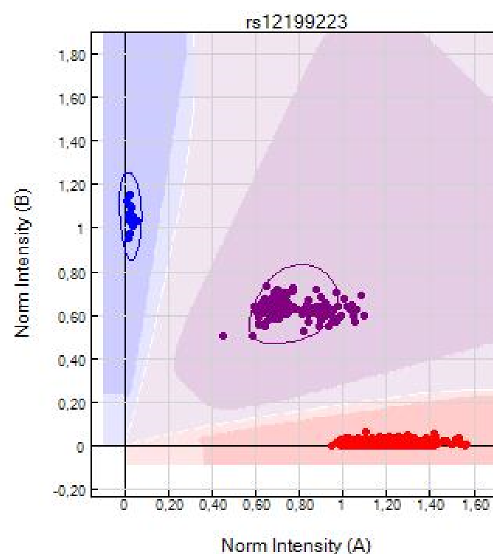
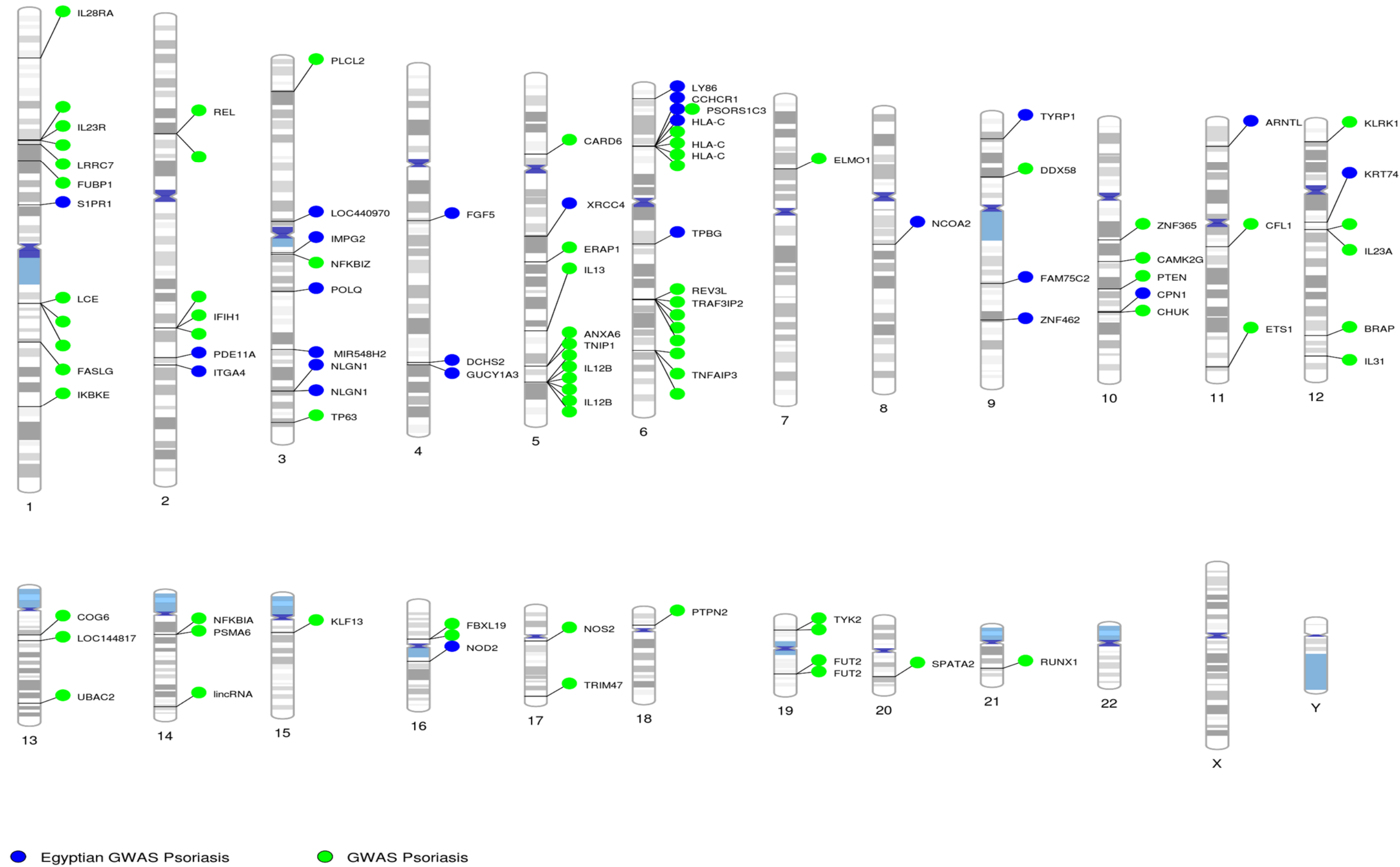


Figure 29 represents the results of the 26 selected SNPs for replication along with the Known SNPs to be associated with psoriasis disease published in the GWAS The NHGRI-EBI Catalog of published genome-wide association studies mapped on chromosomes using the software tool provided by Ritchie lab

Figure 29: Idiogram representing the 26 SNPs selected for replication in blue color and the SNPs replicated in previous GWAS. GWAS Psoriasis: SNPs present in precedent study Egyptian GWAS:SNPs present in our Egyptian Cohort



6. Replication Phase

In the replication phase, all three SNPs located within the MHC-region showed significant association (Table 10). In fact, combined analysis (P_{comb}) of the discovery and replication cohorts revealed that two of these SNPs exceeded the significance threshold for genome-wide association: rs12199223 (P=5.80$\times 10^{-10}$, $P_{\mathrm{comb}=}$6.57$\times 10^{-18}$, OR=2.49, $CI_{95\%}$=2.01-3.1) and rs1265181 (P=1.22$\times 10^{-5}$, $P_{comb=}$1.03$\times 10^{-10}$, OR=2.00, $CI_{95\%}$=1.62-2.45).

Out of the 23 non-MHC SNPs genotyped in the replication phase (Table 10), five SNPs achieved nominal significance with a *P*-value of< 0.05 in replication cohort in which three reached suggestive significance in the combined cohort: rs3770019(P_{comb}=1.02$\times 10^{-05}$, OR=1.55, $CI_{95\%}$=1.30-1.86), rs10960680(P_{comb}=4.72$\times 10^{-7}$, OR=0.97 $CI_{95\%}$=0.69-1.36), and rs10832027 (P_{comb} =7.28$\times 10^{-6}$, OR=1.41, $CI_{95\%}$=1.19 - 1.67) and one SNP rs12650590 reached genome-wide significance (P_{comb}=4.49$\times 10^{-8}$, OR=2.41,$CI_{95\%}$=1.78-3.26).

Table 10: Association evidence for 8 SNPs at 5 loci in GWAS of an Egyptian cohort

Chr: chromosome, RA: reference allele, Freq: Frequency of minor allele, OR: Odd ratio, CI: confidence interval

1. SNPs with P-values below 0.05 in replication phase were considered significant

		SNP	Chr	RA	Freq	Stage	P-value[1]	OR(95%CI)	Gene
MHC	1	rs12199223	6	T	0.15	Discovery	1.96×10^{-09}	2.62(1.91-3.60)	2.8kb 5' of *HLA-C*
						Replication	5.80×10^{-10}	2.32(1.74- 3.09)	
						Combined	$6{,}57\times 10^{-18}$	2.49(2.01 -3.1)	
	2	rs1576	6	G	0.37	Discovery	8.51×10^{-06}	1.76 (1.37-2.26)	*CCHCR1* missense
						Replication	1.93×10^{-07}	3.06(2.27- 4.12)	
						Combined	1.76×10^{-07}	1.67(1.41-1.99)	
	3	rs1265181	6	G	0.14	Discovery	1.83×10^{-05}	1.99 (1.45-2.74)	9.8kb 5' of *HCG27*
						Replication	1.22×10^{-05}	1.90(1.45-2.50)	
						Combined	1.03×10^{-10}	2.00(1.62 -2.45)	
Non-MHC	1	rs3770019	2	T	0.21	Discovery	6.30×10^{-05}	1.76 (1.34-2.33)	*PDE11A* intronic
						Replication	0.017	1.41(1.10-1.80)	
						Combined	1.02×10^{-05}	1.55(1.30-1.86)	
	2	rs12650590	4	G	0.06	Discovery	4.30×10^{-05}	2.54 (1.63-3.98)	48kb 5' of *GUCY1A3*
						Replication	0.0005	2.23(1.48 to 3.37)	
						Combined	4.49×10^{-08}	2.41(1.78-3.26)	
	3	rs1352714	4	G	0.11	Discovery	6.40×10^{-05}	1.99 (1.42-2.8)	*DCHS2*

						Replication	0.01	0.84 (0.60-1.18)	missense
						Combined	9.69×10^{-03}	1.18(0.91-1.52)	
	4	rs10960680	9	A	0.14	Discovery	2.98×10^{-06}	2.01 (1.50-2.70)	209kb 5' of TYRP1
						Replication	0.022	0.97(0.69-1.36)	
						Combined	4.72×10^{-07}	1.50(1.19- 1.89)	
	5	rs10832027	11	A	0.28	Discovery	3.37×10^{-5}	1.68 (1.32-2.15)	*ARNTL* intronic
						Replication	0.026	1.18(0.94- 1.47)	
						Combined	7.28×10^{-06}	1.41(1.19 - 1.67)	

Using LD available we allocated gene near the replicated SNPs. This was done using SNiPa. As we can depict in Figure 30-Figure 34 the genes that lie within or next to the SNP showing a nominal *P*-value in the replication phase.

Figure 30: rs3770019 Linkage disequilibrium plots showing the sentinel variant and its surrounding variants.
rs3770019 is an intronic SNP in Phosphodiesterase 11A (*PDE11A*) gene
The y-axis shows the correlation coefficient (r2) on the left and the recombination rate on the right, the x-axis shows the chromosomal position of each SNP, using SNiPA.

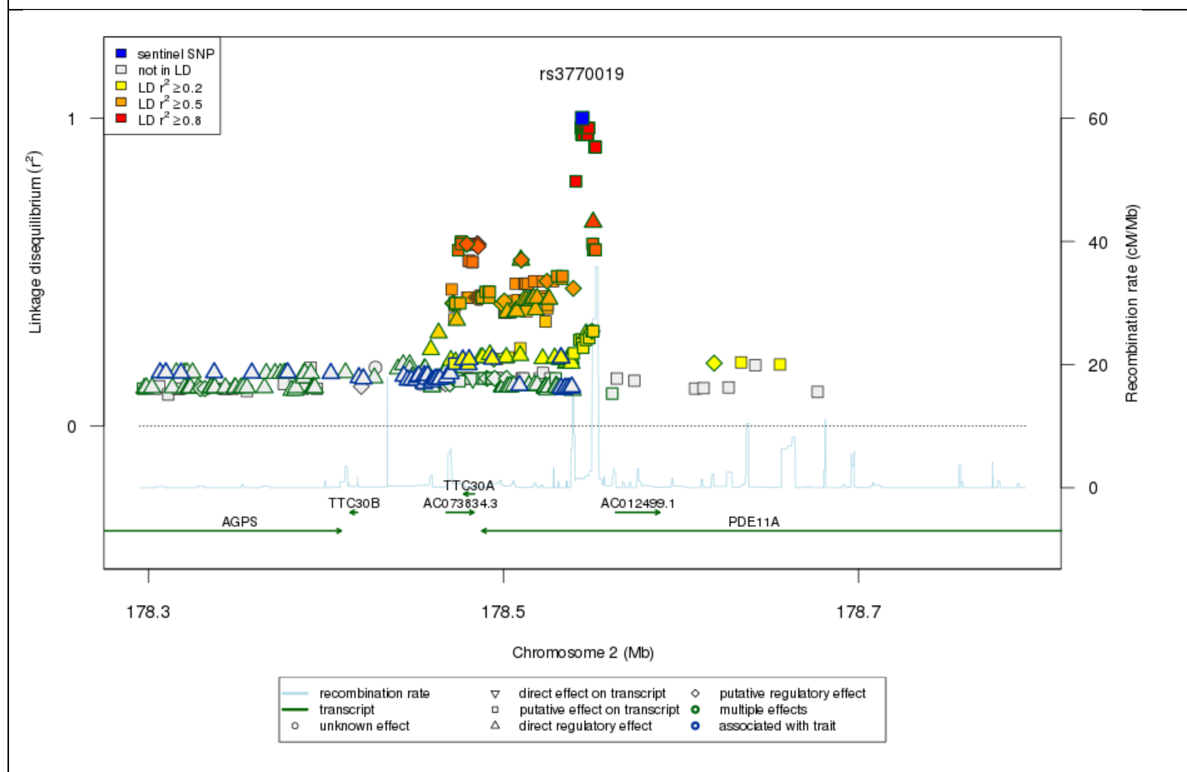


Figure 31: rs12650590 Linkage disequilibrium plots showing the sentinel variant and its surrounding variants.

rs12650590 is 48kb 5' of Guanylate cyclase soluble subunit alpha-3 (GUCY1A3)

The y-axis shows the correlation coefficient (r2) on the left and the recombination rate on the right, the x-axis shows the chromosomal position of each SNP, using SNiPA.

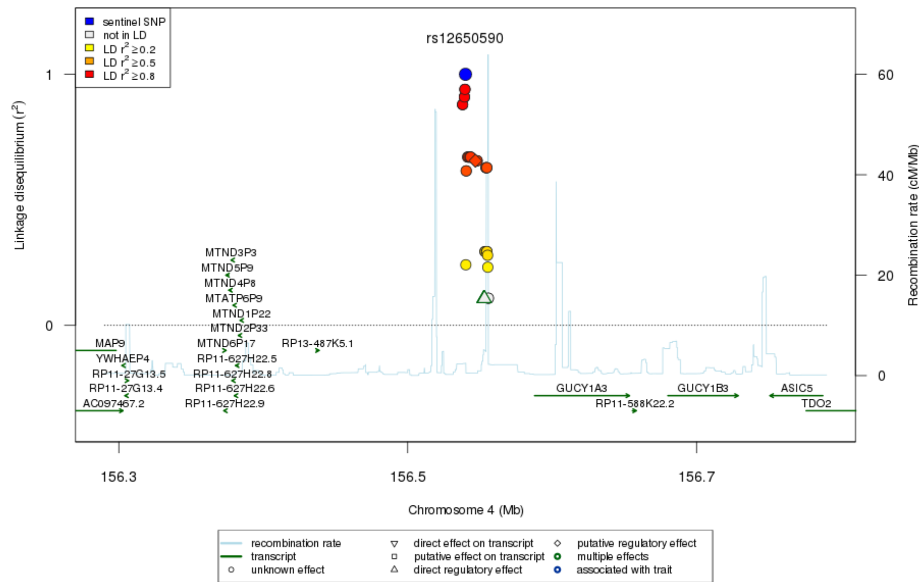


Figure 32: rs1352714 Linkage disequilibrium plots showing the sentinel variant and its surrounding variants.

rs1352714 is missense mutation in Dachsous Cadherin-Related 2 (DCHS2)

The y-axis shows the correlation coefficient (r2) on the left and the recombination rate on the right, the x-axis shows the chromosomal position of each SNP, using SNiPA.

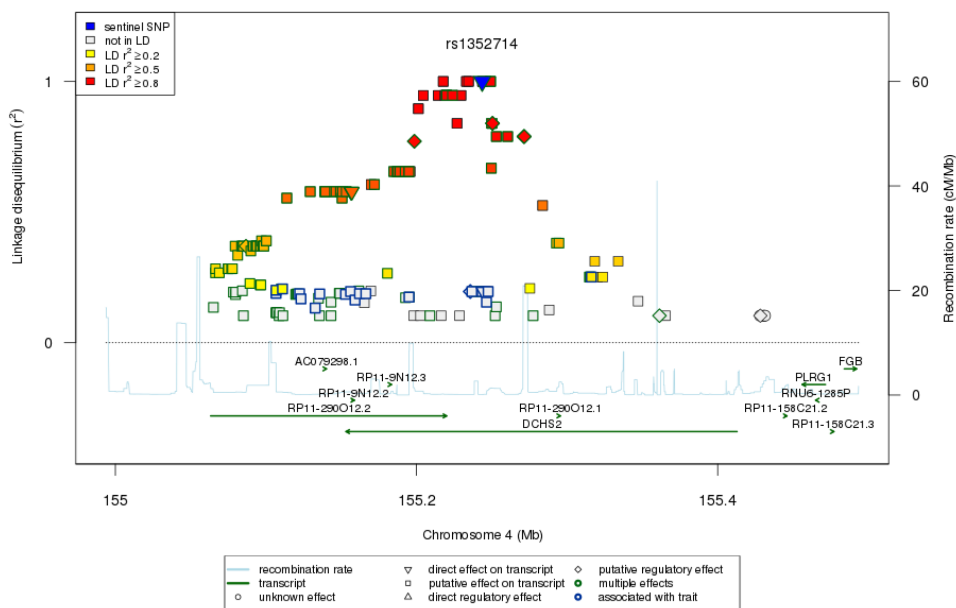


Figure 33: rs10960680 Linkage disequilibrium showing the sentinel variant and its surrounding variants. rs10960680 is 209kb 5' of Tyrosinase-Related Protein 1 (TYRP1) .The y-axis shows the correlation coefficient (r2) on the left and the recombination rate on the right, the x-axis shows the chromosomal position of each SNP, using SNiPA.

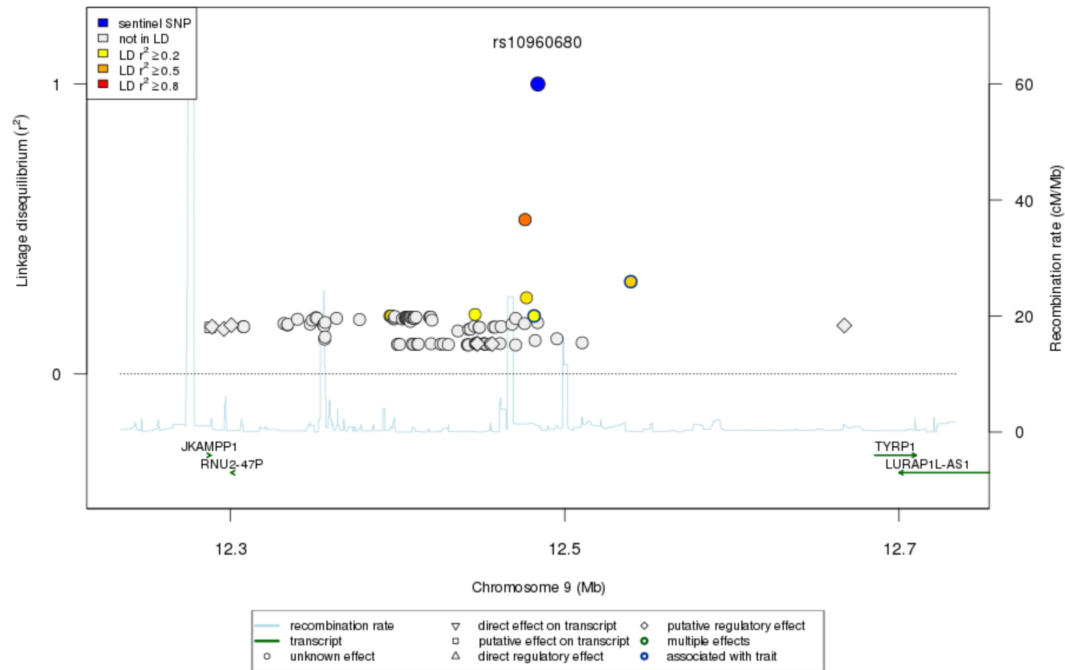
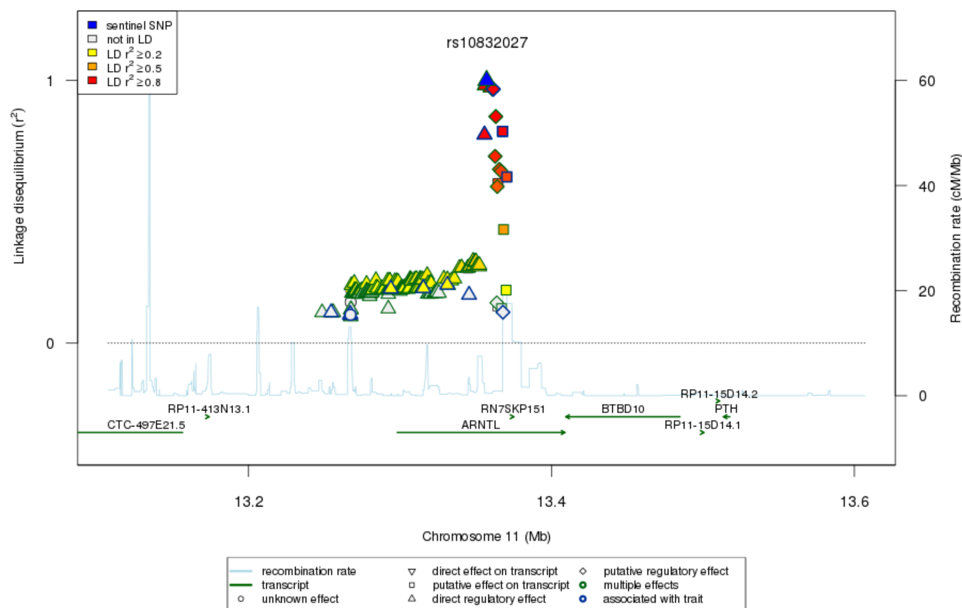


Figure 34: rs10832027 Linkage disequilibrium plots showing the sentinel variant and its surrounding variants. rs10832027 is an intronic variation in Aryl Hydrocarbon Receptor Nuclear Translocator Like (ARNTL). The y-axis shows the correlation coefficient (r2) on the left and the recombination rate on the right, the x-axis shows the chromosomal position of each SNP, using SNiPA.



The below table illustrates the results of all 26 SNPs replicated along their chromosome position, risk allele, odd ratio and genes associated with the SNPs.

Table 11: Association results of the 26 replicated SNPs in an Egyptian cohort

Chr: chromosome, RA: Reference allele, Freq: Frequency of minor allele, OR: Odd ratio, CI: confidence interval

		SNP	Chr	RA	Freq	Stage	P-value	OR(CI $_{95\%}$)	Gene
MHC-locus	1	rs12199223	6	T	0.15	Discovery	1.96×10^{-09}	2.62(1.91-3.60)	2.8kb 5' of *HLA-C*
						Replication	5.80×10^{-10}	2.32(1.74- 3.09)	
						Combined	$6{,}57\times 10^{-18}$	2.49(2.01 -3.1)	
	2	rs1576	6	G	0.37	Discovery	8.51×10^{-06}	1.76 (1.37-2.26)	*CCHCR1* missense
						Replication	1.93×10^{-07}	3.06(2.27- 4.12)	
						Combined	1.76×10^{-07}	1.67(1.41-1.99)	
	3	rs1265181	6	G	0.14	Discovery	1.83×10^{-05}	1.99 (1.45-2.74)	9.8kb 5' of *HCG27*
						Replication	1.22×10^{-05}	1.90(1.45-2.50)	
						Combined	1.03×10^{-10}	2.00(1.62 -2.45)	
Non-MHC	1	rs7552136	1	C	0.12	Discovery	1.10×10^{-05}	2.19 (1.54-3.10)	86kb 5' of *S1PR1*
						Replication	0.19	0.97(0.71 - 1.33)	
						Combined	1.00×10^{-02}	1.40(1.11- 1.76)	
	2	rs35322532	2	T	0.05	Discovery	2.98×10^{-06}	3.42 (2.07-5.64)	*ITGA4* missense
						Replication	0.12	0.41(0.17- 0.96)	
						Combined	1.24×10^{-03}	2.07(1.35- 3.18)	
	3	rs3770019	2	T	0.21	Discovery	6.30×10^{-05}	1.76 (1.34-2.33)	*PDE11A* intronic
						Replication	0.017	1.41(1.10-1.80)	
						Combined	1.02×10^{-05}	1.55(1.30-1.86)	
	4	rs1402216	3	A	0.08	Discovery	1.47×10^{-05}	2.42 (1.62-3.61)	64kb 5' of *NLGN1*
						Replication	0.13	1.44(0.99 to 2.09)	
						Combined	$5{,}45\times 10^{-05}$	1.83 (1.39-2.39)	
	5	rs1484231	3	C	0.30	Discovery	1.80×10^{-05}	0.56 (0.42-0.73)	49kb 3' of *IMPG2*
						Replication	0.33	0.93(0.74-1.17)	
						Combined	9.56×10^{-04}	0.73(0.61- 0.87)	
	6	rs55890443	3	A	0.09	Discovery	1.82×10^{-05}	2.37 (1.60-3.51)	46kb 5' of *NLGN1*
						Replication	0.18	1.39(0.96-2.00)	
						Combined	1.07×10^{-04}	1.77(1.35-2.30)	

	7	rs62255511	3	T	0.09	Discovery	6.40×10^{-05}	2.21 (1.50-3.27)	*LOC440970* intronic
						Replication	0.54	1.19(0.86-1.64)	
						Combined	1.05×10^{-03}	1.57(1.22 to 2.00)	
	8	rs77526264	3	A	0.27	Discovery	7.00×10^{-05}	1.69 (1.31-2.19)	34kb 5' of *MIR548H2*
						Replication	0.65	1.08 (0.86- 1.36)	
						Combined	3.96×10^{-03}	1.32(1.12 to 1.57)	
	9	rs3218634	3	G	0.12	Discovery	6.89×10^{-07}	2.28 (1.65-3.16)	*POLQ* missense
						Replication	0.33	0.81 (0.56- 1.18)	
						Combined	0.056	1.44(1.12-1.85)	
	10	rs12650590	4	G	0.06	Discovery	4.30×10^{-05}	2.54 (1.63-3.98)	48kb 5' of *GUCY1A3*
						Replication	0.0005	2.23(1.48 to 3.37)	
						Combined	4.49×10^{-08}	2.41(1.78-3.26)	
	11	rs1352714	4	C	0.11	Discovery	6.40×10^{-05}	1.99 (1.42-2.8)	*DCHS2* missense
						Replication	0.01	0.84 (0.60-1.18)	
						Combined	9.69×10^{-03}	1.18(0.91-1.52)	
	12	rs16998073	4	A	0.18	Discovery	4.14×10^{-06}	1.97 (1.50-2.63)	3.4kb 5' of *FGF5*
						Replication	0.17	1.03(0.79- 1.34)	
						Combined	$4{,}25\times 10^{-03}$	1.39(1.14-1.68)	
	13	rs7706361	5	T	0.09	Discovery	6.80×10^{-05}	2.23 (1.50-3.31)	35kb 3' of *XRCC4*
						Replication	0.075	1.01(0.72-1.41)	
						Combined	1.26×10^{-03}	1.43(1.11 -1.84)	
	14	rs9328377	6	T	0.22	Discovery	4.40×10^{-07}	2.09 (1.57-2.77)	*LY86* intronic
						Replication	0.19	1.08(0.85 - 1.38)	
						Combined	3.62×10^{-05}	1.43(1.19-1.71)	
	15	rs285982	6	A	0.40	Discovery	8.73×10^{-05}	1.61 (1.27-2.051)	234kb 3' of *TPBG*
						Replication	0.75	1.01(0.81-1.24)	
						Combined	9.90×10^{-02}	1.20(1.01- 1.42)	
	16	rs16936870	8	T	0.10	Discovery	2.60×10^{-05}	2.08 (1.48-2.90)	*NCOA2* intronic
						Replication	0.41	1.08(0.76- 1.53)	
						Combined	$2{,}14\times 10^{-03}$	1.54(1.20- 1.98)	
	17	rs10960680	9	A	0.14	Discovery	2.98×10^{-06}	2.01 (1.50-2.70)	209kb 5' of TYRP1
						Replication	0.022	0.97(0.69-1.36)	
						Combined	4.72×10^{-07}	1.50(1.19- 1.89)	

	18	rs4480216	9	C	0.10	Discovery	7.50×10^{-06}	1.49 (1.186-1.89)	59kb 3' of *FAM75C2*
						Replication	0.29	1.28(0.94-1.75)	
						Combined	1.23×10^{-04}	1.28 (1.0-1.64)	
	19	rs7031901	9	G	0.08	Discovery	4.50×10^{-05}	2.38 (1.57-3.60)	*ZNF462* intronic
						Replication	0.34	0.98(0.67-1.43)	
						Combined	1.36×10^{-02}	1.46(1.10-1.92)	
	20	rs11599750	10	C	0.47	Discovery	2.60×10^{-05}	1.69 (1.32-2.15)	*CPN1* intronic
						Replication	0.32	1.18(0.96-1.46)	
						Combined	3.93×10^{-04}	1.35(1.16 to 1.58)	
	21	rs10832027	11	A	0.28	Discovery	3.37×10^{-5}	1.68 (1.32-2.15)	*ARNTL* intronic
						Replication	0.026	1.18(0.94- 1.47)	
						Combined	7.28×10^{-06}	1.41(1.19 - 1.67)	
	22	rs11170177	12	G	0.05	Discovery	$1{,}29\times 10^{-05}$	2.73 (1.74-4.28)	*KRT74* missense
						Replication	0,45	1.28(0.66 to 2.4)	
						Combined	$1{,}43\times 10^{-05}$	2.29(1.54 to 3.40)	
	23	rs2066845	16	G	0.05	Discovery	8.80×10^{-06}	2.96(1.83-4.76)	*NOD2* missense
						Replication	0.58	1.01(0.54-1.87	
						Combined	5.03×10^{-05}	2.23(1.50- 3.33)	

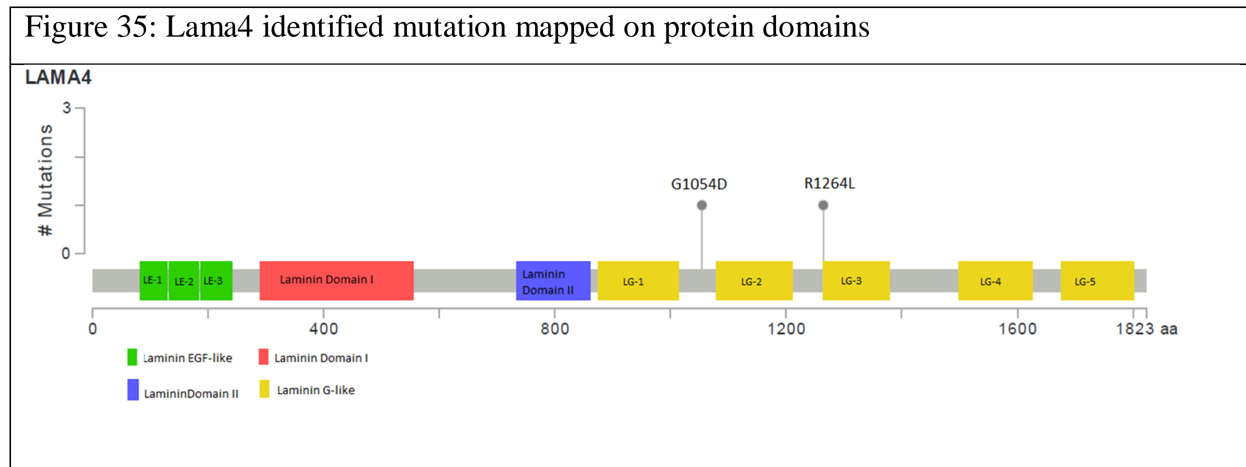
Familial Psoriasis

1. Exome Sequencing and Sanger sequence validation

A mean of 5-9 Gb of sequence was generated per individual. The cumulative and sequencing depths of the sequenced samples met the requirements of 50X depth. After Data alignment and duplicate remover of the sequencing, variants found in controls and affected individuals were filtered out. Synonymous changes were far less likely to be causative, they were also filtered out. Exome sequencing identified 160,811 variants on average on both families' diseased subjects of which 39,207 missense and splicing variants. After applying filtration on the remained variant, 10 nonsynonymous mutations in Family I and 4 in Family II were left (Table 12, Table 13). Both unrelated families shared a mutation in Laminin α4 chain at different positions; a heterozygous mutation c.G3161A/ p.G1054D in family I, and c.G3791T/p.R1257L in family II, both predicted to be deleterious variants by SIFT software.

Mutation in Family I a substitution from glycine to aspartic acid was mapped between laminin globular domain LG-1 and LG-2 Mutation in Family II a substitution from arginine to leucine was mapped upstream to laminin Globular domain LG-3 (Figure 35).

Figure 35: Lama4 identified mutation mapped on protein domains



Sanger sequencing validation was performed on all 11 studied family members and both variations were validated and segregated within each family.

Table 12: Data filtration of exome sequencing Family I

D: damaging, T: tolerated, P: probably damaging, B: benign, AA: Amino acid

Gene	AA Change	SIFT	Polyphen
ANK1	p.V420M	D	D

LAMA4	**p.G1054D**	**D**	**D**
IRF9	p.G72A	D	D
ANKRD29	p.E77V	T	D
AHDC1	p.Q1395K	D	P
KLHL6	p.P491S	D	P
ERI2	p.G272A	.	D
SRCAP	p.R319L	T	P
RAD54L	p.S486T	T	B
MED12L	p.P218S	D	B

Table 13: Data filtration of exome sequencing Family II AA: Amino acid, D: damaging, T: tolerated, P: probably damaging			
Gene	AA Change	SIFT	Polyphen
PREX1	M1015T	D	P
NEIL1	p.R425W	D	P
TRMT6	p.K319	D	D
LAMA4	**p.R1257L**	**D**	**P**

2. Targeted resequencing of the LAMA4-locus

The set of exome sequence derived suspected causal mutations in Laminin α4 (LAMA4) gene. The sequenced target region of 784,424 bp covering the LAMA4 in 100 psoriatic sporadic cases and 92 controls samples resulted in a cluster density of 1000-1400 K/mm2 and the average passing filter was 85%. Both identified mutations were absent in all sequenced samples. Gene-wise analysis results using SKAT are depicted in Table 14. The table illustrates the gene spanning the targeted sequenced region along with their P value. Although a non-significant *P*-value in LAMA4 gene *P* =0.17 indicating no association with psoriasis, a suggestive significant *P*-value was shown in Tube1 gene *P*=0.01.

Tube 1 gene effect was further investigated using co-expression analysis by looking at the genes co-regulated across multiple tissues and experiments. Figure 36 and Figure 37 depict the results from a Gene Set Enrichment Analysis on the correlation values. The results are shown in Figure 36 that Up-regulated GO Terms include mitochondrial function, cell cycle, DNA replication. The GO Terms from anti-correlated genes relate to immune response and inflammation (Figure 37).

Table 14: Gene wise analysis using SKAT software
Showing no association of *LAMA4* with psoriasis and a suggestive association in *TUBE1*

SetID	**P.value**	**N.Marker.All**	**N.Marker.Test**
FAM229B	0.153773941	133	131
FYN	0.522201113	1208	1203
LAMA4	0.17890482	960	954
RNU6-1226P	0.591682694	62	62
RP1-142L7.5	0.053044595	130	130
RP1-142L7.8	0.079603431	63	62
RP1-142L7.9	0.145218111	84	84
RP1-97J1.2	0.869360836	49	49
RP11-506B6.6	0.009589666	171	167
RP3-415N12.1	0.15566279	40	37
TRAF3IP2	0.45367942	283	283
TRAF3IP2-AS1	0.066783568	539	534
TUBE1	0.014045342	114	114
WISP3	0.116146675	143	141
snoU13	0.71519408	104	99

Figure 36: Positively correlated Go Terms for Gene Set Enrichment Analysis on Tube1 gene

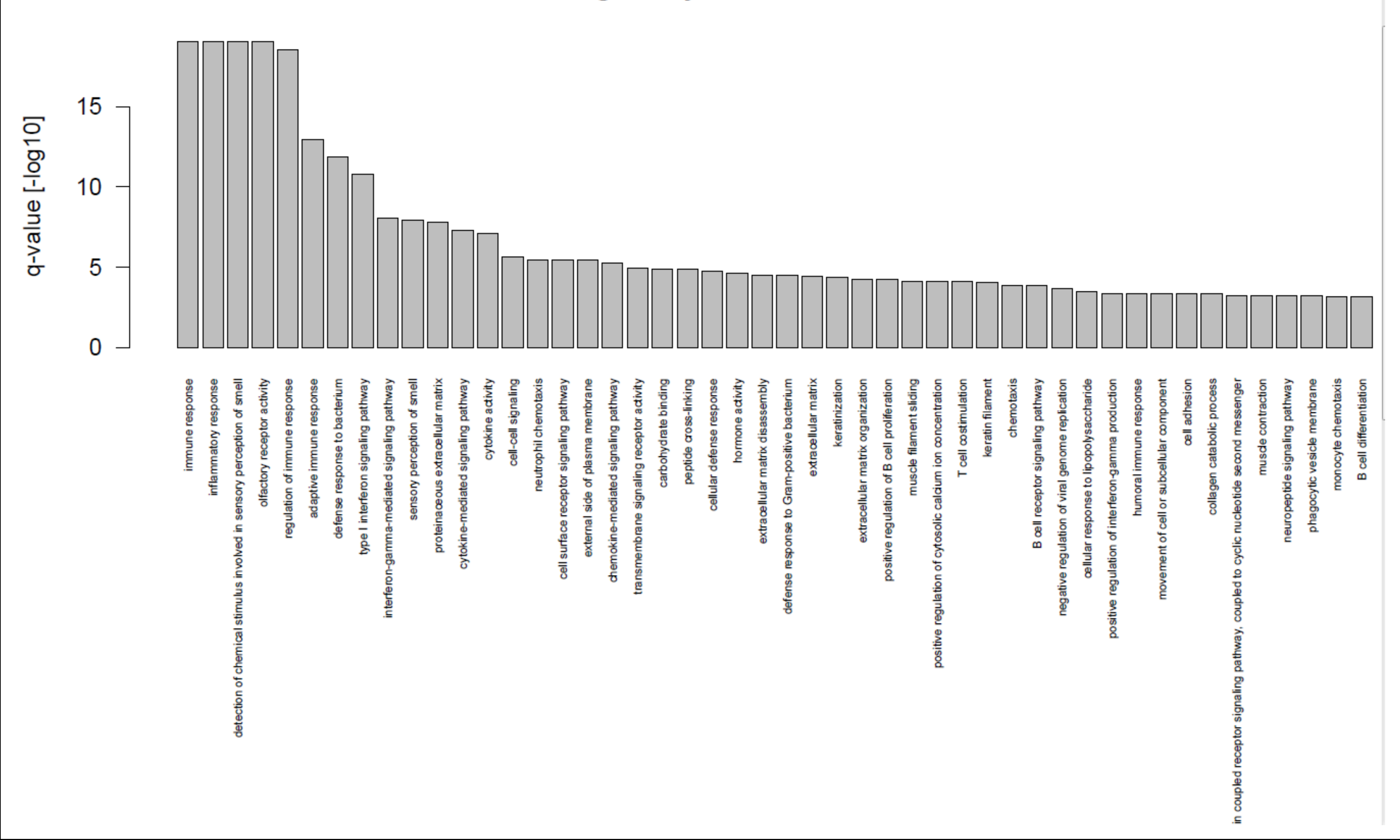
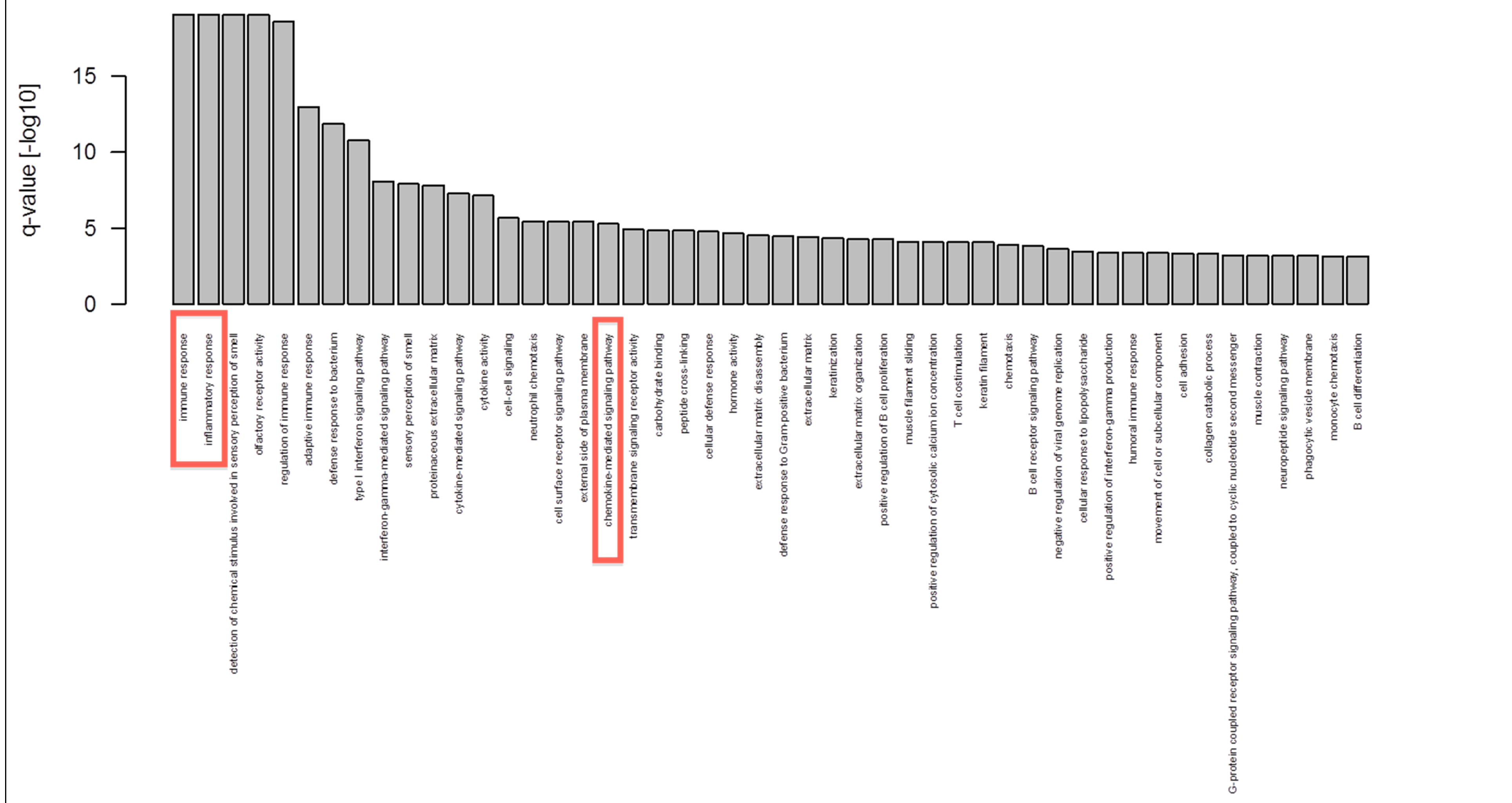


Figure 37: Negatively correlated Go Terms for Gene Set Enrichment Analysis on Tube1 gene

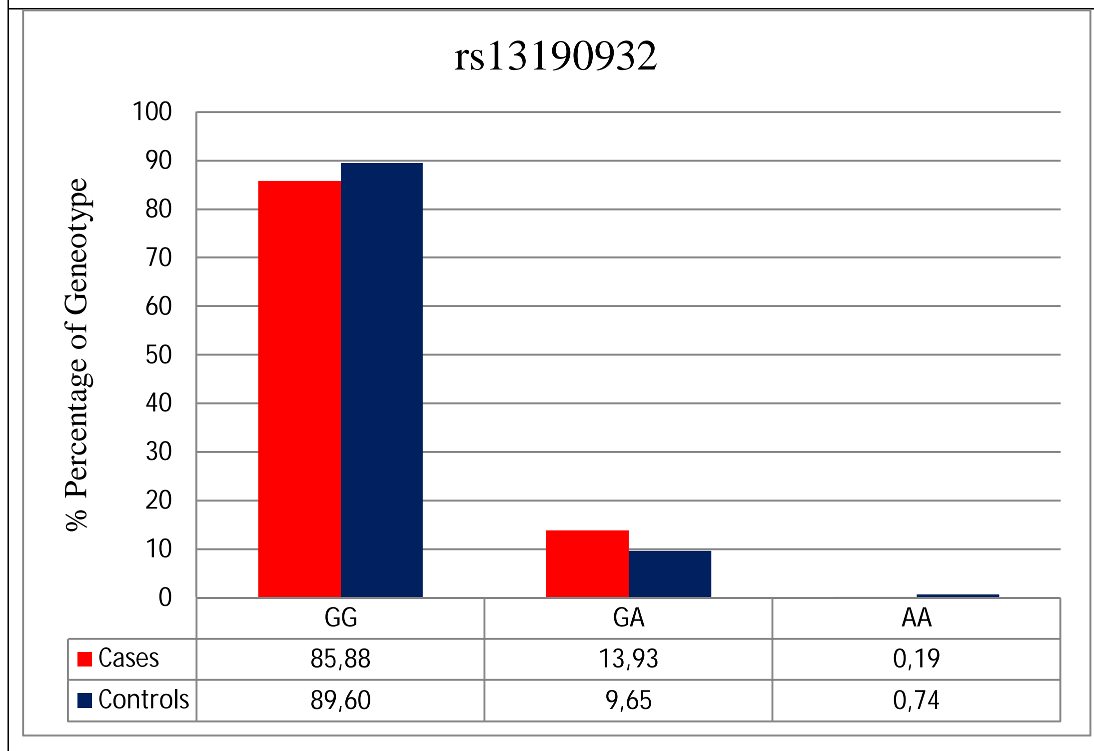


3. Replication of the *TRAF3IP2* SNPs in other cohorts using Taqman

Two SNPs in TRA3IP2 gene were genotyped. rs13190932 in 524 cases and 808 control showed χ^2 value of 0.023 (OR=1.3070,CI95= 0.95- 1.79) however rs3398055 was not significance with a *P*value=0.3.

Figure 38: rs13190932 TRA3IP2 in Egyptian population

In 524 cases and 808 controls *P*value=0.023



Mitochondrial sequence analysis

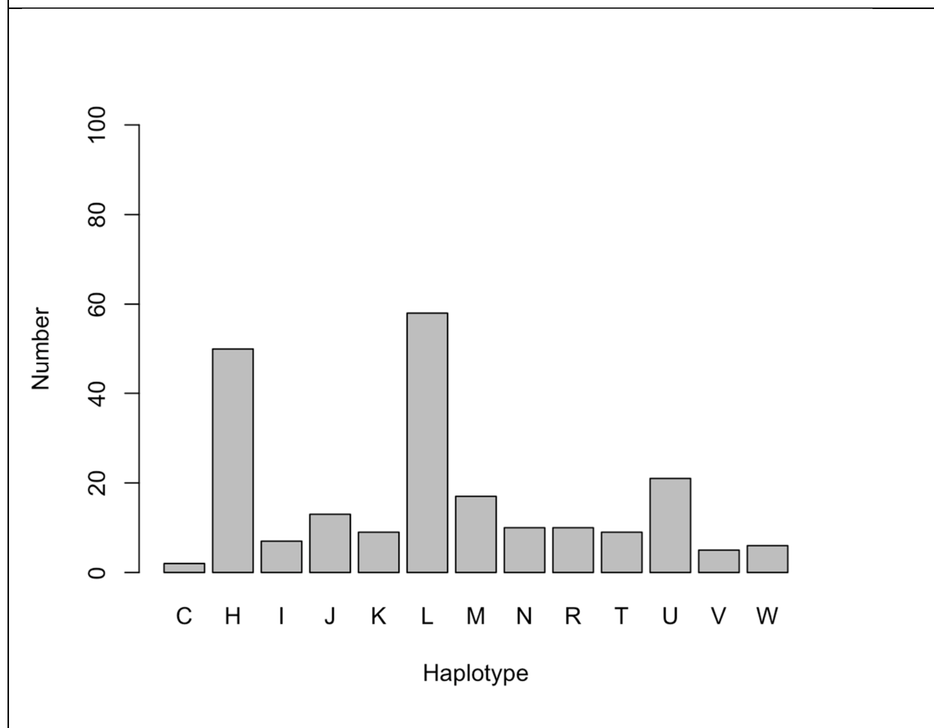
Sequence analysis of the entire mtDNA of 110 psoriatic patients and 107 controls detected 1,010 SNPs after the removal of non-significant ones. Of the remaining 1,010 SNPs: 74 SNPs considered common and the remaining 919 as rare.

The following criteria were applied to define a rare SNP: SNPs with a variant allele frequency of 2% and counted less than 15X in the reads were called as rare SNPs.

The distribution of mtDNA haplogroups analyzed in all samples is shown in Figure 39. The most dominant haplogroup in modern Egyptian population are the H and L haplogroups.

Figure 39:mtDNA haplogroups distribution within samples

X-axis: Number is showing the absolute counts, Y-axis is the haplotype.



When we stratified the haplogroups data according to cases and controls, we can depict some differences in the haplogroup distribution (Figure 40). As seen in Figure 40psoriatic patients have a tendency to carry more Haplogroups U and M comparing to control samples. This tendency is also seen when applying principle component analysis on the haplogroup information; however, due to the low number of samples, this can’t be conclusive.

Table 15: Distribution of haplogroups within cases and controls, absolute counts.

	C	H	I	J	K	L	M	N	R	T	U	V	W
control	2	28	3	5	3	31	6	8	4	5	7	1	4
Psoriasis	0	22	4	8	6	27	11	2	6	4	14	4	2

Figure 40: Relative distribution of haplogroups compared to Psoriasis and controls, relative counts.

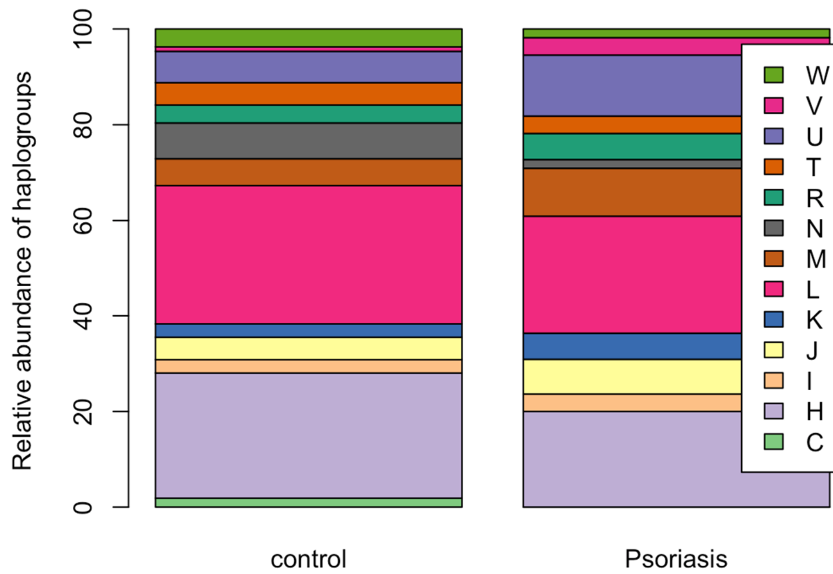
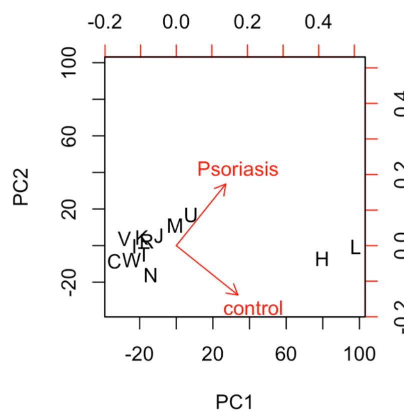


Figure 41: For comparison of Control and Psoriasis patients we run the following Principle Component Analysis on the haplogroup information



Due to the small number of samples, we combined haplogroups that are phylogenetically associated. We can still see a trend of an increase in the number of haplogroup M-C, U-K in addition to R and J-T in cases. Applying a χ^2 test in each haplogroup between cases and controls showed that the N haplogroup is more prevalent in control samples with a P=0.05 this haplogroup might play a protective role (Figure 42).

Figure 42: Haplogroup distribution in percentage between control and cases

red bar represent cases, blue bar represents control samples

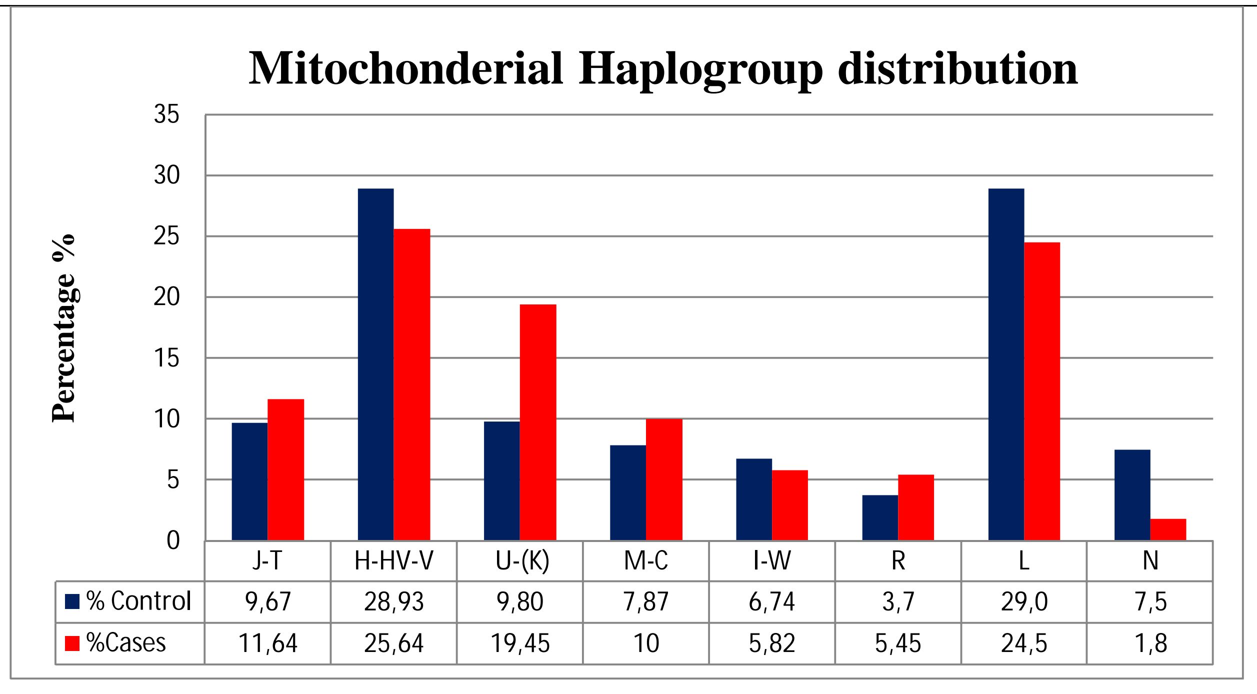


Table 16: χ^2 test between cases and controls in each haplogroup

Haplogroups	Control n=110	Cases n=107	*P*-value
J-T	10	12	0.72
H-HV-V	29	2	0.61
U-(K)	10	20	0.08
M-C	8	11	0.53
I-W	7	6	0.74
R	4	6	0.56
L	31	27	0.53
N	8	2	0.05

We performed SNPwise analysis using the Fisher exact test on 1158 SNPs, 6 SNPs showed significance *P*-value less than 0,05. Two of these SNPs fall in RNR2 gene, two in the D-loop

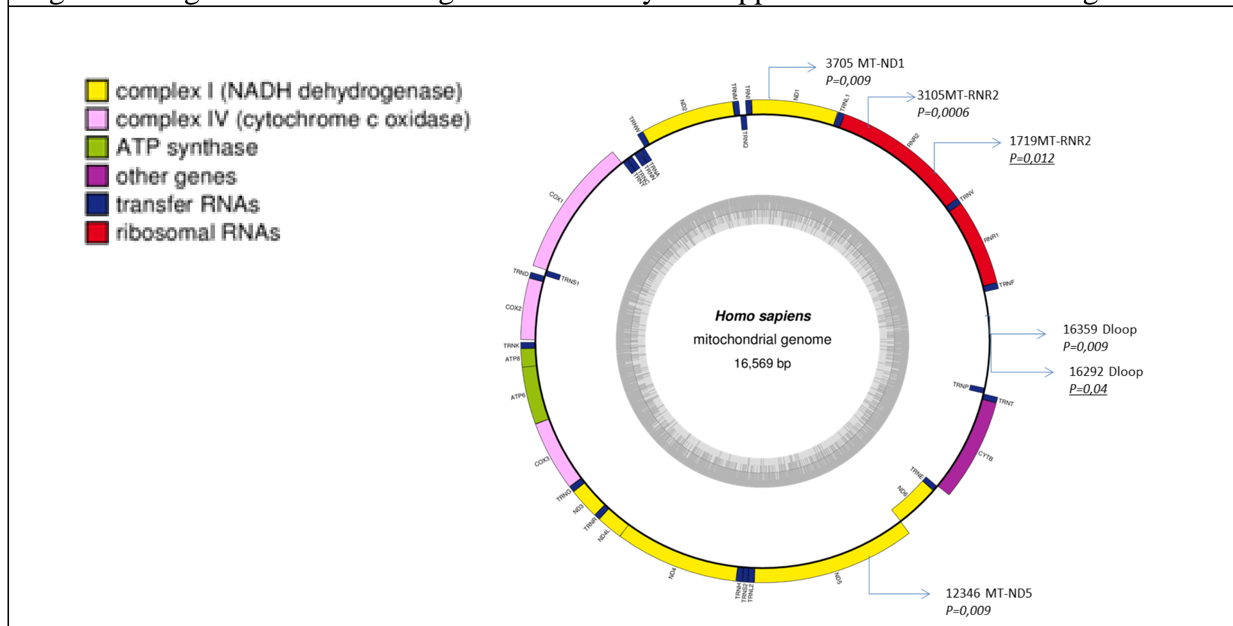
region, one in the ND1 and another in ND5. Table 17 summaries the SNPs location and *P*-values. Figure (37) depicts these SNPs on the mitochondria sequence.

Table 17: SNPwise analysis for mitochondrial for SNPs with *P*-value <0,05

*Combined Annotation Dependent Depletion (CADD)

	SNP position	Allele change	Locus	Gene	Change effect	CADD phred *	Pvalue
1	1719 (rs3928305)	G/A	16S	MT-RNR2	Non-coding transcript exon	4.921	0.0112
2	3105	A/C	16S	MT-RNR2	Non-coding transcript exon	4.745	0.0006
3	3705	G/A	ND1	MT-ND1	synonymous Leucine=>Leucine	4.567	0.0099
4	12346	C/T	ND5	MT-ND5	Non-synonymous H=>Y	4.999	0.0099
5	16292 (rs38682930)	C/T	MT-HV1	D-Loop	Non-coding	5.277	0.0478
6	16359 (rs370567324)	T/C	MT-HV1	D-Loop	Non-coding	4.001	0.0099

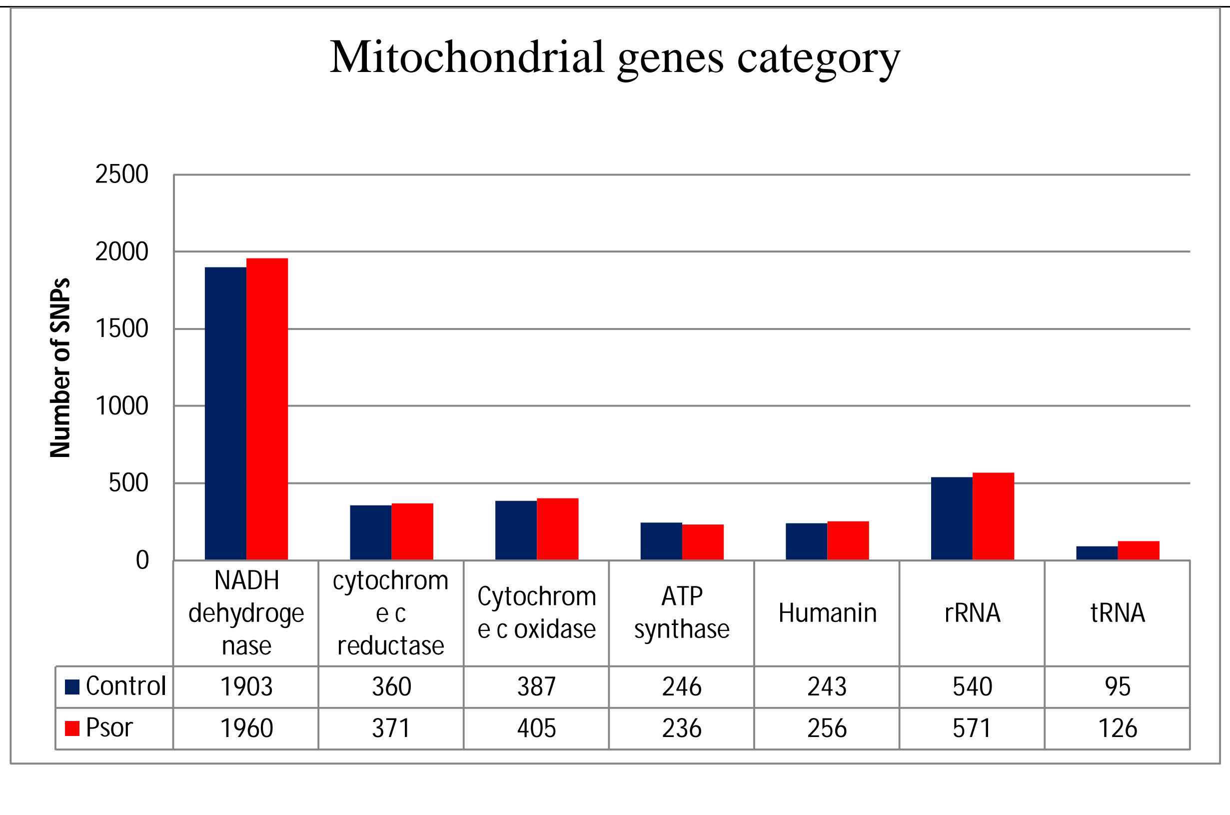
Figure 43: significant SNPs using SNPwise analysis mapped on the mitochondrial genome



This prompted us to look in more detail at the gene level, as depicted in Figure (38)

Figure 44: Genewise analysis according to the gene function within the mitochondria

Psor: psoriatic samples



	NADH dehydrogenase	cytochrome c reductase	Cytochrome c oxidase	ATP synthase	Humanin	rRNA	tRNA
Control	1903	360	387	246	243	540	95
Psor	1960	371	405	236	256	571	126

Figure 44 shows the accumulation of variations within genes in psoriatic patients comparing to controls. Although the statistically this is not significant, we can't disregard that this might have an effect on the mitochondrion function in general.

Discussion

Genome-Wide Association

Linkage and association studies have demonstrated that the MHC region represents the major genetic determinant for psoriasis susceptibility. In accordance with previous studies (Tsoi et al., 2012), our present data show a strong association of the MHC-locus with psoriasis, with rs12199223 being the most significant disease marker in which HLA-C 0602 was determined as the risk allele by the HLA imputation results from the 'HIBAG' R package. This is in line with the data generated by the fine mapping of MHC associations in a European population with psoriasis where they indicated that HLA-C0602 has the strongest association in psoriasis (Okada et al., 2014)(Knight et al., 2012). Another SNP located in the MHC-region that we found to be associated with psoriasis was rs1576, a non-synonymous SNP at *CCHCR1*. rs1576 was indicated as a genetic risk factor for psoriasis in previous linkage and association studies (Tervaniemi et al., 2012) and *CCHCR1* was shown to be highly polymorphic and differently expressed in psoriatic skin (Elomaa et al., 2003).

The third SNP located in the MHC-region, that we found to be associated, was rs1265181. This SNP had previously been shown to be associated with psoriasis in the European and Chinese cohort (Zhang et al., 2009). Association of the MHC locus with psoriasis in our Egyptian population adds evidence to the role of innate and adaptive immunity in the disease etiology (Bowcock, 2005).

In accordance with previous findings by Haase et al. (Haase et al., 2014), no signal was detected within the IL23R and IL12B genes although one interpretation of this finding is the limited power of the study. The association evidence within the TRAF3IP2 locus, a gene that encodes ACT1, a signaling adaptor involved in the regulation of adaptive immunity (Hüffmeier et al., 2010b), were shown to be consistent with allelic heterogeneity between the Caucasian and Egyptian population. There were two independent associations at rs33980500 and rs13190932 in the Caucasian and Chinese samples (P=1.13×10^{-20} and1.03×10^{-8}respectively), however only one association was significant at rs76228616 (P=1.29 × 10^{-4}, OR=1.15) among Egyptians.

Analyzing associated SNPs located outside the MHC-region, we could identify pathways of considerable biological relevance to psoriasis pathogenesis from which rs10832027, an intronic mutation in the Aryl Hydrocarbon Receptor Nuclear Translocator Like (ARNTL)

gene, was enriched in the circadian rhythm pathway. Ando et al. showed that expression of core clock genes such as *Per2* and *Bmal1* (*ARNTL*) showed circadian rhythms in the wild-type, but not in *Clock*-mutated mouse skin, suggesting that the Clock gene is a novel regulator of psoriasis-like skin inflammation in mice via direct modulation of *IL-23R* expression in TCRγ/δ^+ T cells, establishing a mechanistic link between psoriasis and the circadian clock (Ando et al., 2015).In line, employees on night shifts have an increased risk of psoriasis (Li et al., 2013), suggesting that circadian disruption may contribute to psoriasis-development through dysregulation of the immune system (Li et al., 2013)(Plikus et al., 2015). Additionally, an experimental model of psoriasis in BALB/cJ mice showed increased levels of proinflammatory cytokines in response to paradoxical sleep deprivation (Hirotsu et al., 2012).

Another SNP showing significance in our study is rs10960680 (P_{comb}= 4.72× 10^{-07}), located 209kb 5' of Tyrosinase-Related Protein 1(*TYRP*), a gene involved in melanin synthesis shown to be significantly down-regulated in lesional and non-lesional skin of psoriasis samples compared with healthy subjects (Loite et al., 2013).

We further found an association of the locus 4q32.1 at marker rs12650590 48kb 5' of *GUCY1A3* with a P_{comb}=4.49× 10^{-08}. Matthews *et al.* reported a locus for familial psoriasis mapping to chromosome 4, which was designated as *PSORS3* (Matthews et al., 1996). Another marker, rs1352714 at chromosome 4, showed a tendency toward association with a P_{comb}=9.69× 10^{-03}. rs1352714 is a missense mutation at Dachsous Cadherin-Related 2 (*DCHS2*), a gene coding for a Calcium-dependent cell-adhesion protein, which had previously been shown to be associated to the age of onset of Alzheimer's disease (The Alzheimer’s Disease Neuroimaging Initiative et al., 2012).
A novel association was also replicated at locus 2q31.2 at marker rs3770019 (P_{comb}=1.02× 10^{-05}). Chromosome 2 had only been shown to be associated with psoriasis in a GWAS study conducted by Trembath *et al,* where they identified associated regions on chromosome 2 (*D2S134*) using a total of 106 affected sibling pairs identified from 68 independent families (Trembath et al., 1997b).

The 5 non HLA SNPs with suggestive association found in our data were checked in the latest large meta-analysis of Tsoi et al. (Tsoi et al., 2017) which consisted of 5 GWAS of European Caucasian descent, however, none of these SNPs showed significant association in the meta-analysis. This can be explained by the ethnic differences between the two populations.

Further refinement and functional characterization of these association signals hold the promise to generate significant mechanistic insights into the dysregulation of immune responses in psoriasis. Interestingly, preliminary analysis of pathways enriched in our study is in compliance with earlier studies. Several studies showed the involvement of PPARα in the mechanism of epidermal barrier development and its proliferation and differentiation (Hanley et al., 1999). Kömüves *et al.* pointed out that PPARα ligands promote the differentiation and can restore homeostasis in hyperproliferative mouse skin in addition to the regulation of epidermal apoptosis. (Kömüves et al., 2000) Additionally PPARs have been shown to regulate T-cell survival, activation, and $CD4^+$ T cell differentiation (Choi and Bothwell, 2012) PPAR agonist ligands have been implicated in inhibiting the production of IL-17 and IFNγ by CD4 $^+$T cells presenting a relevant source of research in the treatment of psoriasis (Lima et al., 2013).

Another enriched pathway is the PI3Kpathway, It has been shown that Imiquimod-induced skin inflammation is attenuated in PI3Kγ deficient and PI3Kδ kinase-dead mice. Also, inhibition of PI3Kδ using IC87114 and PI3Kγ using AS605240 and AS614006 reduced pro-inflammatory cytokine secretion in human $CD4^+$ memory T cells and peripheral blood mononuclear cell from psoriatic patients (Roller et al., 2012).

In summary, we identified novel candidate SNPs for psoriasis in Egyptians. Outside the MHC-region, there was no overlap between the significantly associated SNPs we identified and those previously identified loci in other ethnicities. Therefore, our study suggests that there is an inter-ethnic genetic difference in psoriasis-susceptibility. Moreover, pathways enriched in our study affirm the role of genes involved in T cell activation and differentiation in the prognosis of the disease.

While the possibility of the low statistic power due to the limited sample size compared to the previously reported studies cannot be excluded, differential genetic associations could also indicate an environmental impact (e.g. climate, nutrition or host microbiota) on the pathogenesis of psoriasis. Egyptians are exposed to an environment, which varies from those Europeans and Asians inhabit, where most known disease-associated variants were reported. In fact, there is growing evidence that environmental factors contribute to psoriasis (Barrea et al., 2016)(Dika et al., 2007). Identification of ethnicity-specific candidate genes and variable environmental factors will advance our current understanding of the complex pathogenesis of psoriasis.

Familial Psoriasis

Here, we report for the first time two novel nonsense mutations p.G1054D, p.R1257L in the *LAMA4* in PsV affected members of the unrelated family I and II respectively. Both mutations led to a damaging likelihood using Sift software and had an effect on the amino acid polarity; where the p.G1054D led to a change from a non-polar amino acid to polar in Family I and the p.R1257L let to a polar to on polar change in Family II.

The targeted resequencing results of the LAMA4 gene showed the absence of the identified mutations in 100 psoriatic sporadic cases and 92 controls suggesting that the identified mutations are family specific in our data set. This was also confirmed by the results from the target re-sequenced region where genewise analysis using SKAT on *LAMA4* was not significant.

SNPs in TRAF3IP2, a gene 3' 662Kb distal from LAMA4, was associated with psoriasis in the German population. In concordance with previous data, rs13190932 was significant with a *P*-value of 0.023 in 524 cases and 808 control genotyped samples. This shows that PsV sporadic genetic subjects in the Egyptian population share rs13190932 association with German Population while different in ethnicity (Ellinghaus et al., 2010a).

The laminin α4 chain is part of large heterotrimers consisting of α, β, and γ polypeptide chains (Colognato and Yurchenco, 2000). It is a component of laminin-8 (α4:β1:γ1, 411) and laminin-9 (α4:β2:γ1, 421)(Miner et al., 1997). It is widely expressed in vascular endothelial basement membrane (BM) (Iivanainen et al., 1997) and has a significant role in normal blood vessel maturation (Thyboll et al., 2002). Investigations have suggested that laminin α4 supports T cell migration through the vessel wall, as these cells tend to extravasate preferentially at sites where laminin α4 is expressed (Sixt et al., 2001). Mutations in the LAMA4 gene were associated with severe dilated cardiomyopathy. Knoll et al. (2007) sequenced the LAMA4 gene in 180 Caucasian patients and identified a nonsense (R1073X) and a missense (P943L) mutation in 2 diseased subjects (Knoll et al., 2007).

This is the first time LAMA4 mutation is associated with PsV disease. PsV is a disorder of both innate and adaptive immune systems, in which keratinocytes, dendritic cells, and T cells have central roles. Key mediators in the disease process include infiltration of TH cells, particularly TH17 cells, and overproduction of proinflammatory cytokines and create an inflammatory environment(Boehncke and Schön, 2015). Leukocyte recruitment is a key

component in inflammatory reactions. For leukocytes to penetrate the vessel wall and migrate to the extravascular tissue, they need to interact sequentially with endothelial junctional components and the perivascular basement membrane (Nourshargh and Marelli-Berg, 2005). Any disturbance in the basement membrane can lead to incorrect leukocyte recruitment or inflammation process. Several studies in LAMA4 null and wild-type mice determined that the lack of laminin-411 in the perivascular BM influences extravasation of inflammatory cells. It was previously reported that null LAMA4 mice exhibit reduced accumulation of polymorphonuclear leukocytes in inflamed tissue(Wondimu, 2004). Recent data indicated that T cell infiltration into the central nervous system in experimental autoimmune encephalomyelitis is reduced in LAMA4 null mice (Wu et al., 2009).

Recently two different approaches were used to stimulate leukocyte extravasation in the mouse ear skin, the croton oil which triggers an unspecific inflammatory reaction that involves recruitment of different leukocyte subclasses and a delayed-type hypersensitivity reaction which was induced in the ear skin by local challenge subsequent to sensitization with Fluoro-2,4-dinitrobenzene. Response to both approaches found that the ratio between right and left ear weight was significantly smaller in the Lam4 null mice compared with WT, indicating reduced immune cell recruitment (Kenne et al., 2010). Additionally, it was shown that laminin α4 is required for effective monocyte and polymorphonuclear leukocytes extravasation using the air pouch model stimulated by the monocyte chemoattractant MCP-1 and Thioglycollate-induced pleurisy respectively (Kenne et al., 2010).

CD18 hypomorphic PL/J mouse a mouse model that develops a skin disease that closely resembles human psoriasis. However, it was shown that the same mutation on C57BL/6J background did not exhibit the psoriasiform phenotype. It was demonstrated that an introduction of a 9-centimorgan fragment of chromosome 10 derived from the PL/J strain into the disease-resistant CD18 hypomorphic C57BL/6J is promoting the development of psoriasiform skin disease, this fragment is comparable with to the human chromosome 6q (Wang et al., 2008). Looking closely at the genes present with the identified region we can remark that LAMA4 gene is one of potential candidate genes (Figure 45).

Figure 45: Potential candidate genes in the interval of chromosome 10 in congenic mice compared with a syntenic map of human chromosome 6q. Adapted from: (Wang et al., 2008).

CM 0 21 22 23 24 25 26 27 28 29 30 31 (chromosome 10)

Mouse symbol	Human symbol	Human Location	Mouse gene name
Alcp15			Alcohol preference locus 15, male specific
Alcp16			Alcohol preference locus 16, female specific
Pas11			Pulmonary adenoma sussceptibility 11
Braf-rs1			
Col10a1	COL10A	6q21-q22	Procollagen, type X, alpha 1
Marcks	MARCKS	6q22.2	Myristoylated alanine rich protein kinase C substrate
Echdc1	ECHDC1	6q22.33	Enoyl Coenzyme A hydratase domain containing 1
Tspy14	TSPYL4	6q22.1	TSPY-like 4
Odc-rs19			Ornithine decarboxylase, related sequence 19
Lith21			Lithogenic gene 21
Mpmv40			Modified polytropic murine leukemia virus 40
Rps17-rs14			Ribosomal protein S17, related sequence 14
Xmv54			Xenotropic murine leukemia virus 54
Amd1	AMD1	6q21-q22	S-adenosylmethionine decarboxylase 1
Cd164	CD164	6q21	CD164 antigen
Cfid			Cystic fibrosis intestinal distress
Crsp3	CRSP3	6q22.33-q24.1	Cofactor required for Sp1 transcriptional activation, subunit 3
Fcs4			Fibroblast cell senescence 4
Fyn	FYN	6q21	Fyn proto-oncogene
Gpr6	GPR6	6q21	G protein-coupled receptor 6
lama4	LAMA4	6q21	Laminin, alpha 4
Sec63	SEC63	6q21	SEC63-like (S. cerevisiae)
Sesn1	SESN1	6q21	Sestrin 1
Wasf1	WASF1	6q21-q22	WASP family 1
Lace1	LACE1	6q22.1	Lactation elevated 1
Nr2e1	NR2E1	6q21	Nuclear receptor subfamily 2, group E, member 1
Snx3	SNX3	6q21	Sorting nexin 3
Atg5	ATG5	6q21	Autophagy-related 5 (yeast)
Cd24a	CD24	6q21	CD24a antigen
Hdac2	HDAC2	6q21	Histone deacetylase 2
Rplp2-rs1			Ribosomal protein, large P2, related sequence 1
Tpi-rs6			Triosephosphate isomerase related sequence 6
Sim1	SIM1	6q16.3-q21	Single-minded homolog 1 (Drosophila)
Grik2	GRIK2	6q16.3-q21	PR domain containing 1, with ZNF domain
Prdm1	GRIK2	6q16.3-q21	Glutamate receptor, ionotropic, kainate 2 (beta 2)
Ros1	ROS1	6q22	Ros1 proto-oncogene
Prep	PREP	6q22	Prolyl endopeptidase
Gja1	GJA1	6q21-q23.2	Gap junction membrane channel protein alpha 1
Aim1	AIM1	6q21	Absent in melanoma 1
Bves	BVES	6q21	Blood vessel epicardial substance
Popdc3	POPDC3	6q21	Popeye domain containing 3
Rtn4ip1	RTN4IP1	6q21	Reticulon 4 interacting protein 1
Ostm1	OSTM1	6q21	Osteopetrosis associated transmembrane protein 1
Pkib	PKIB	6q22.3	Protein kinase inhibitor beta, cAMP dependent, testis specific
Foxo3a	OSTM1	6q21	Forkhead box O3a
Pdss2	PDSS2	6q21	Prenyl (solanesyl) diphosphate synthase, subunit 2

TH17 cells play an essential role in different autoimmune diseases such as psoriasis and multiple sclerosis (MS) and facilitate different pathogenic responses. A study has identified the melanoma cell adhesion molecule (MCAM) ligand as laminin 411 suggesting that MCAM and laminin 411 interact to facilitate TH17 cell entry into tissues and promote inflammation(Flanagan et al., 2012, p. 411). Monoclonal antibodies (mAbs) to the laminin α4 globular domain are able to inhibit tumor cell adhesion and migration on laminins 411 and 421, and that both α6β1 integrin and MCAM bind α4-laminins at very close sites on the globular domain (Ishikawa et al., 2014)

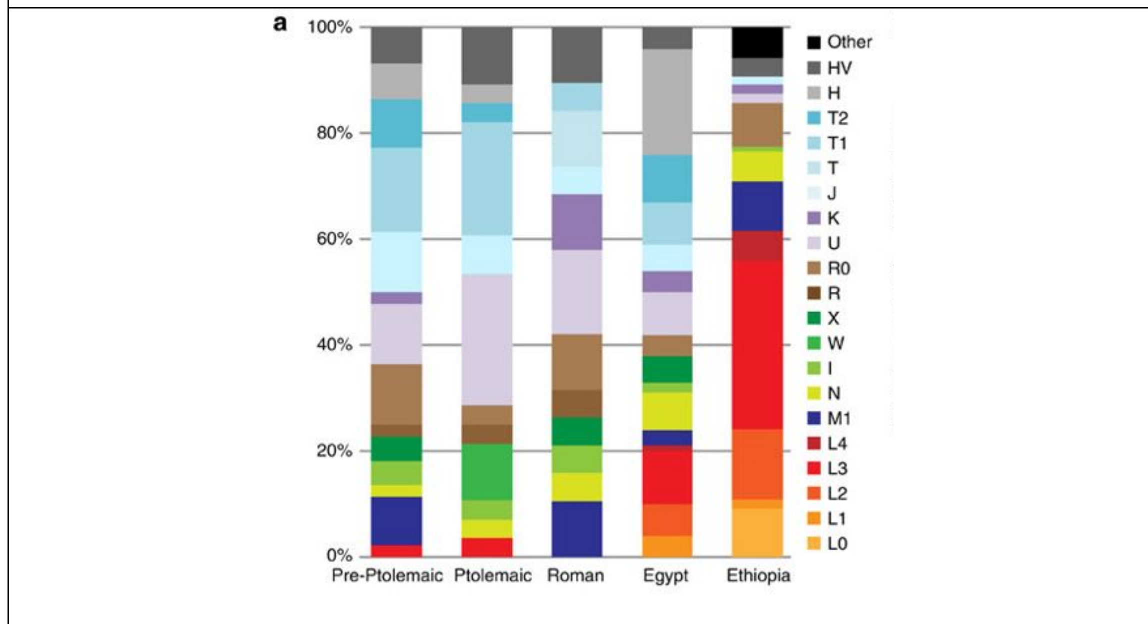
Both mutations were located within highly conserved areas of the molecule and within the 1st third globular domains which may affect its interaction with integrin and MCAM.in addition, all mutations were found in patients affected with PsV and not in any unaffected individuals or even psoriatic sporadic cases showing that these mutations are family specific. Further investigation of mutations effect should be performed to elucidate the function and effect in vivo.

In summary, several lines of evidence point that both mutations in *LAMA4* found in our study play a plausible role in disease manifestation and seems to possess a gain of function. The future plan is to induce psoriasis in a knock out LAMA4 mice using Imiquimod and Il-23 and investigate its role in the disease manifestation.

Mitochondrial haplogroup and SNP association

This study investigated the distribution of mtDNA haplogroups in 107 healthy Egyptians and110 psoriatic cases with disease severity from moderate to severe and the absence of psoriasis arthritis. In general, we observed highly similar haplogroup profiles between our data and the earlier published data on modern Egyptian population by Schenemann Figure 46, where they depicted the dominance of the Haplogroup L and H (Schuenemann et al., 2017).

Figure 46: Mitochondrial DNA haplogroup frequencies of three ancient populations Pre-Ptolemaic, Plotemaic and Roam and two modern-day populations Egyptian and Ethiopian. Adapted from (Schuenemann et al., 2017)



Haplogroup L represents the African mtDNA lineage (Cerezo et al., 2012) where the H haplogroup is predominantly in Europe and also present in North Africans (Ennafaa et al., 2009).

Analyzing the distribution of haplogroup in cases and controls, we can see that psoriatic patients have a tendency to carry more Haplogroup U and M compared to control samples, and due to the small number of samples, we combined the haplogroups that are phylogenetically associated and yet we can still see the same trend of increase in the number of haplogroup M-C, U-K in addition to R and J-T in cases compared to controls. Some of these haplogroups were associated with different diseases such as Haplogroup T was found to be a risk factor for developing peripheral neuropathy during antiretroviral therapy (Castro et al., 2006) and age-related macular degeneration(Canter et al., 2008; SanGiovanni et al., 2009).

Applying a χ^2 tests in each haplogroup between cases and controls we found significance in the haplogroup N with a *P*-value of 0.05, N haplogroup was under-represented in the cases suggesting a protective mechanism. N haplogroup is derived from the L3 mtDNA African lineage (González et al., 2007). The mitochondrial DNA haplogroup N was shown to contribute to the good survival of gastric cancer patients compared to the mitochondrial DNA haplogroup M. This study has shown that macrohaplogroup M exhibited higher respiratory activity than haplogroup N this is explained by the higher mtDNA content, mtRNA transcript levels, and complex III abundance in haplogroup N. It was demonstrated that haplogroup M had higher reactive oxygen species levels and NAD^+/NADH in comparison to haplogroup N (Zhou et al., 2017).

mtDNA haplogroups and their characteristic SNPs can be advantageous or detrimental. They have been implicated in a number of diseases and pathological conditions, including cancer (Shen et al., 2010), aging (Ren et al., 2008), diabetes (Lu et al., 2010), osteoarthritis (Fang et al., 2014), schizophrenia (Bi et al., 2016), and Leber's hereditary optic neuropathy(Sudoyo et al., 2002). A phylogenetic tree of L3 subhaplogroups that migrated from Africa to East Asia revealed the association of many of the subclades with metabolic and degenerative diseases (Kuo et al., 2016; Niu et al., 2015). The link between mtDNA haplogroup/SNPs and disease has been attributed to possible changes in mitochondrial reactive oxygen species (ROS) levels, respiration capacity, and ATP production (Fang et al., 2014; Gómez-Durán et al., 2010). Up to date, only one study was conducted to test the hypothesis of an association between mtDNA variants and psoriasis and psoriasis arthritis in the Spanish population. No haplogroup was significantly associated with the risk for psoriasis (Coto-Segura et al., 2012). This may be due to the low power of the study.

Our results of the SNPwise analysis using Fisher exact test resulted in 6 different variations with nominal significance. Two of these variations are G1719A and A3105C in Mt-RNR2 with *P*value of 0.01 and 0.0006 respectively. Mt-RNR2 is transcribed into the 16S mitochondrial ribosomal RNA (rRNA) and harbors a short ORF that is translated into the 24-amino acid humanin (HN) peptide (Kearsey and Craig, 1981; Guo et al., 2003). 16S RNA plays different roles; it is considered as a neuroprotective factor where it suppresses apoptosis by binding to BAX and prevents the translocation of BAX from the cytosol to mitochondria.

At the position m.1719, control samples were showing homoplasy to the other allele G comparing to the cases suggesting protective machinery. Another significant variant is a non-

synonymous variation is G3705A in the MT-ND1. It is subunit 1, one of 7 mitochondrial DNA (mtDNA) encoded subunits among the respiratory Complex I NADH ubiquinone oxidoreductase (Brink et al., 1987). It plays a role in the first step in the electron transport chain of mitochondrial oxidative phosphorylation (OXPHOS) and is located within the mitochondrial inner membrane. This variation was detected in ovarian carcinomas (Liu et al., 2001).

A significant missense mutation at MT-ND5 was detected at C12346T. This variation will lead to an amino change from histidine to tyrosine. MT-ND5 is mitochondrially encoded NADH dehydrogenase subunit 5 which is also part of complex I. It was also detected in patients with Leber hereditary optic neuropathy (LHON)-like optic neuropathies (Abu-Amero and Bosley, 2006).

The last two significant variations were detected in D-loop at position C16292T and T16359C. The non-coding D-loop region is involved in many functions including replication, transcription, and organization of the mitochondrial genome. Its name ‘D-loop’ is derived from ‘displacement loop’ and it is also called the control region. It is an early replication intermediate in which replication of the H-strand starts at a fixed point (oriH) also several RNAs are transcribed from both H- and L-strands. The D-loop is a 1 kb in length and lies between two tRNA genes on mtDNA. (Yamamoto, 2001). It has been established that D-loop mutations resulting in instability of the mitochondrial genome. Although mutations occur throughout the entire mitochondrial genome, D-loop is the most variable region of the human mitochondrial genome (Parsons et al., 1997). T16359C was detected in two of 152 endometriosis patients and absent in 150 controls (Govatati et al., 2013) however C16292T was not detected in any disease.

Aberrant mitochondrial structure and function effect tissue homeostasis a contributing to multiple human disorders and aging. Around 10% of patients with primary mitochondrial disorders present skin manifestations such as hair abnormalities, rashes, pigmentation abnormalities, and acrocyanosis. Minor attention has been paid that some skin diseases are linked to alterations of mitochondrial energy metabolism (Feichtinger et al., 2014a). The speculation is that the mechanisms include impairment in respiratory function, an increase in the production of oxygen-free radicals, and apoptosis. Further replication and increase in sample size is planned for future confirmation of the discovered results, and to further study the function of the identified variations.

Conclusion

Psoriasis is a complex disease of the skin. A comprehensive understanding of how its pathogenesis and how it develops will require studies of both genetic and environmental factors. Disease susceptibility was shown to vary between different ethnic groups in this thesis. We attempted to study for the first time the Egyptian population by exploring the genetic underpinnings of this disease through genome-wide association studies to identify genetic risk factors, and by whole exome sequencing of affected members of unrelated families, in addition, we investigated the correlation of mitochondrial variants and haplogroup distribution with the disease. Our results from the genome-wide association study (GWAS) in an Egyptian population showed concordance with the findings from previous studies on other populations, where we found a genome-wide significant association between the MHC locus and the disease at rs12199223 (P_{comb}= 6.57×10^{-18}) and rs1265181 (P_{comb}=1.03×10^{-10}). However, we identified a novel significant association with the disease at locus, 4q32.1 (rs12650590, P_{comb}=4.49×10^{-08}) in the vicinity of gene GUCY1A3, and multiple suggestive associations, e.g. rs10832027 (P_{comb}=7.28×10^{-06}) and rs3770019 (P_{comb}=1.02×10^{-05}). This suggests the existence of important inter-ethnic genetic differences in psoriasis susceptibility without disregarding the difference in the environment between the populations. Future plans are to investigate the skin microbiota in the Egyptian population to understand the effect of environment in the disease pathogenesis.

The whole-exome sequencing of the two independent Egyptian families resulted in the discovery of two novel nonsense mutations p.G1054D, p.R1257L in the laminin alpha 4 gene *LAMA4* in psoriasis vulgaris affected members of family I and II respectively. This was followed by a targeted resequencing for a region of 784,424 bp covering the *LAMA4* and *TRAF3IP2* region at chromosome 6q21 by screening 100 psoriatic and 92 control Egyptian samples. This showed the absence of the identified mutations in the screened samples suggesting that the identified mutations are family specific. This was further confirmed by the results from the target re-sequenced region where gene-wise analysis using SKAT of *LAMA4* was not significant. In concordance with previous data rs13190932, a SNP in *TRAF3IP2* 3'662 Kb distal from LAMA4, was associated with psoriasis in the German population, showed a suggestive significance with a *P* value of 0.023 in 524 cases and 808 control genotyped samples. An animal experiment is planned to further study the effect of the knock out of the *LAMA4* in mice in the pathogenesis of psoriasis using the Il23 as an inducer of the disease.

The whole genome mitochondria sequencing of 107 healthy Egyptians and110 psoriatic cases showed a trend in the distribution of phylogenetically combined haplogroups, an increase in the number of haplogroup M-C, U-K in addition to R and J-T in cases compared to controls. χ^2 tests in each haplogroup between cases and controls showed a significance in haplogroup N with a *P*-value of 0.05, N haplogroup was under-represented in the cases suggesting a protective mechanism. The SNPwise resulted in 6 different variations with nominal significance. Two of these variations are G1719A and A3105C in Mt-RNR2 with *P*value of 0.01 and 0.0006 respectively. Two located in the D loop at position m.16292 and m.16359 with a *P*-value of 0.04 and 0.009 respectively. In addition a non-synonymous variation at m.12346 located in MT-ND5 and another at m.3705 at MT-ND1. Further investigation of the function of these variations needs to be carried with an increase in the number of samples in the replication phase to confirm the results.

Studies of genetic of psoriasis in ethnically diverse populations will help us to identify susceptibility loci specific to the population studied in addition will define a narrower boundary of the commonly identified loci. Other to a small association study reporting of psoriasis in Egyptian and Tunisian populations little is known of the genetic basis of psoriasis in North African in general. This is the first comprehensively study in the Egyptian population.

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